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Erythrinaline alkaloids from the flowers and pods of *Erythrina lysistemon* and their DPPH radical scavenging properties

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Abstract

Fourteen different erythrinaline alkaloids have been isolated from the flowers and pods of *Erythrina lysistemon* with four being reported for the first time in nature and five for the first time in this species and the rest having been re-isolated. The new compounds are (+)-11β-hydroxyerysotramidine (**1**), (+)-11β-methoxyerysotramidine (**2**), (+)-11β-hydroxyerysotrine *N*-oxide (**4**) and (+)-11β-hydroxyerysotrine (**8**). (+)-11α-Hydroxyerysotrine *N*-oxide (**3**), earlier misidentified as erythrartine *N*-oxide (β-hydroxyerysotrine *N*-oxide **4**), was also re-isolated along with four other alkaloids. Correct identification of compounds **4** and **8** was aided by the fact that the two sets of C-11 epimers **3**, **4** and **8**, **9** were both isolated in this study thus making it easier to identify and assign the individual epimers. (+)-Erythristemine (**14**) was found distributed in most of the plant parts investigated. Preliminary work on the crude chloroform/methanol (1:1) showed moderate toxicity to brine shrimp (LC_{50} 23 ppm) and moderate (IC_{50} 86 μg/ml) radical scavenging properties against stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The DPPH radical scavenging properties of the isolated compounds were assessed using TLC autographic and spectrophotometric assays whereupon only compounds **11** (1 μg; 90 μg/ml) and **12** (0.1 μg; 160 μg/ml) showed any notable activity. It appears the two compounds are slow reacting and do not reach steady state conditions within the standard half an hour time frame but only seemed to have reached steady state conditions after 4 h.

Keywords: *Erythrina lysistemon*; Fabaceae–Papilioniodeae; Erythrinaline alkaloids; C-11 epimers; Radical scavenging properties

1. Introduction

The genus *Erythrina* comprises of over 110 species of orange or red flowered trees, shrubs and herbaceous plants and is found throughout the tropical and subtropical regions of the world. There are thirty species and subspecies in tropical Africa and six species in Southern Africa (Allen and Allen, 1981; Fabian and Germishhuizen, 1997). *Erythrina lysistemon* Hutch. is a deciduous tree, which grows up to 12 m tall with an open but sparse round reddish crown. It is distributed from Tanzania in the north to Eastern Cape in the south of Africa (Venter and Venter, 1996). The extracts of the leaves, roots and stem bark of this plant have a significant history in traditional medicine where it has been used in the treatment of various ailments which tally with the observed biological activities (NAPRALERT, 2003). Prior to this work 19 erythrinaline alkaloids have been reported from the seeds of this plant (NAPRALERT, 2003; Amer et al., 1991a,b). Also reported are prenylated flavonoids and isoflavonoids from the stem and stem bark (NAPRALERT, 2003; McKee et al., 1997; Obiero, 2001; El-Masry et al., 2002). As part of our continuing investigation of the secondary metabolites of the genus *Erythrina*, we now describe the isolation and structural elucidation of four new erythrinaline alkaloids from the flowers of *E. lysistemon* namely (+)-11β-hydroxyerysotramidine (**1**), (+)-11β-methoxyerysotramidine (**2**), (+)-11β-hydroxyerysotrine *N*-oxide (**4**) [up to now known as erythrartine *N*-oxide] and (+)-11β-hydroxyerysotrine (**8**) while another five, (+)-11α-hydroxyerysotrine *N*-oxide (**3**) [earlier misidentified as erythrartine *N*-oxide, i.e. β-hydroxyerysotrine *N*-oxide],

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(+)-11β-methoxyerysotrine *N*-oxide [(+)-*O*-methylerythrartine *N*-oxide] (**5**), (+)-erythrabine (**6**), (+)-erysotramidine (**7**), 11-α-hydroxyerysotrine (**9**) [earlier misidentified as the 11-β-epimer] were isolated for the first time in *E. lysistemon* in addition to another five, (+)-erysotrine (**10**), (+)-erysodine (**11**), (+)-11α-hydroxyerysodine (**12**), (+)-erysotrine *N*-oxide (**13**) and (+)-erythristemine (**14**) which were re-isolated. Examination of the chloroform and methanol extracts of the pods yielded compounds **1, 4, 8, 12** and **14**. Compound **14** was also observed in the stem wood, twigs and leaves. Preliminary work on the crude chloroform/methanol (1:1) showed moderate toxicity to brine shrimp (LC_{50} 23 ppm) and moderate (IC_{50} 86 μg/ml) radical scavenging properties against stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical.

2. Results and discussion

The chloroform soluble fraction of the 1:1 $CHCl_3$/MeOH extract of the flowers of *E. lysistemon* was separated by a combination of chromatographic procedures and recrystallization to yield 13 erythrinaline alkaloids. Compounds **1** and **2** showed molecular ion peaks at *m*/*z* 343.0 and 357.1, respectively, in their EI-MS spectra, suggesting the molecular formulae $C_{19}H_{21}NO_5$ and $C_{20}H_{23}NO_5$. In their ^{13}C NMR spectra, 19 and 20 non-equivalent carbon signals, respectively, were observed, which was consistent with the EI-MS results. Further from the DEPT and the HMQC data of compound **1**, 12 protonated carbons were detected out of which three were methoxyl, two methylene and seven were methine (two oxymethine). On the other hand, thirteen protonated carbon signals were detected for compound **2** out of which four were methoxyl, two methylene and seven were methine (two oxymethine). The ^1H (Table 2) and ^{13}C NMR data (Table 1) of both compounds **1** and **2** are very similar to those of erysotramidine (Amer et al., 1991a) with the only difference being in the replacement of the methylene signal by an oxymethine signal at the C-11 position. In the ^{13}C NMR, the signal for this moiety appeared downfield at δ 67.2 and 74.4 ppm for compounds **1** and **2**, respectively. In the ^1H NMR spectra of these compounds, the signals for this moiety appeared as *dd* centred at δ 4.51 and 4.77 ppm, respectively. In the HMBC spectra, these protons correlated with the C-12 carbon resonating at δ 128.9 and 127.8 ppm, respectively. Total assignment was done by a close examination of the COSY, HMBC and HMQC data of these compounds. Compound **1** was thus identified as (+)-11β-hydroxyerysotramidine. Compound **2** showed an extra methoxyl group, which could easily be placed at C-11 and was thus identified as (+)-11β-methoxyerysotramidine.

Compounds **3** and **4** were obtained as brownish oily substances and their molecular formulae were suggested to be $C_{19}H_{23}NO_5$ deduced from the APCI MS data, which showed a quasimolecular ion $[M+H]^+$ 345.8. This was consistent with ^{13}C NMR results, which revealed the presence of 19 non-equivalent carbons. Further, from the DEPT and the HMQC data of compound **3** and **4**, 13 protonated carbons were detected out of which three were methoxyl, three were methylene and seven were methine (two oxymethine). The ^1H (Table 2) and the ^{13}C NMR (Table 1) data for these compounds are very similar to those of (+) erysotrine *N*-oxide (Amer et al., 1991a). The only difference is in the replacement

Table 1
^{13}C NMR spectral data of **1**–**5**, **8**, **9** and **14** (75.4 MHz in $CDCl_3^*$ and $CD_3COCD_3^\#$)

	1*	**2***	**3**#	**4**#	**5**#	**8***	**9***	**14***
1	125.2	124.7	125.6	125.6	126.4	126.2	125.9	126.2
2	137.0	137.0	133.5	133.2	133.6	131.6	132.0	131.6
3	75.0	75.0	76.0	76.0	75.8	76.5	76.4	76.4
4	41.8	38.9	31.2	30.9	30.7	40.8	40.6	40.9
5	65.9	65.6	82.2	82.2	82.3	66.6	66.9	66.5
6	157.6	157.8	138.9	138.7	138.5	142.3	142.3	142.3
7	120.4	120.1	119.3	118.8	119.5	124.2	123.8	124.2
8	171.5	167.7	65.2	60.0	60.5	58.5	59.1	58.4
10	44.0	42.5	72.6	72.8	73.6	46.2	51.3	45.6
11	67.2	74.4	64.3	74.5	75.0	73.9	64.9	74.0
12	127.8	128.9	126.4	126.2	123	127.7	128.7	126.8
13	128.8	127.4	126.9	123.3	128	130.1	129.9	130.7
14	107.9	108.2	112.5	107.9	111.8	108.2	109.1	108.9
15	148.8	148.9	149.8	148.9	150.1	145.0	148.8	148.9
16	149.5	149.3	149.8	147.1	149.6	146.8	148.9	148.4
17	112.6	113.4	108.5	115.8	108.4	114.9	110.7	111.6
OCH_3	56.9	56.0	55.9	56.8	57.8	57.9	56.4	56.4
	56.4	55.8	55.6	55.5	56.3	56.3	56.3	56.2
	56.3	55.6	55.4	55.1	56.2	56.2	56.2	56.1
		55.5			56.1			

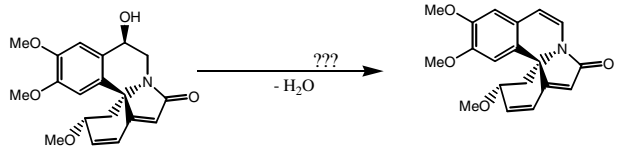
Table 2
^{1}H NMR spectral data of **1**–**5**, **8**, **9** and **14** (300 MHz in $CDCl_3^*$ and $CD_3COCD_3^\#$)

	1*	**2***	**3**$^\#$	**4**$^\#$	**5**$^\#$	**8***	**9***	**14***
1	6.85 (1H, *dd*, $J = 9.4, 2.0$ Hz)	7.01 (1H, *dd*, $J = 10.2, 2.4$ Hz)	6.73 (1H, *dd*, $J = 10.0, 2.1$ Hz)	6.73 (1H, *dd*, $J = 10.0, 2.3$ Hz)	6.59 (1H, *dd*, $J = 10.2, 2.2$ Hz)	6.60 (1H, *dd*, $J = 10.1, 2.2$ Hz)	6.61 (1H, *dd*, $J = 10.0, 2.1$ Hz)	6.60 (1H, *dd*, $J = 10.1, 2.1$ Hz)
2	6.32 (1H, *br d*, $J = 10.1$ Hz)	6.45 (1H, *br d*, $J = 10.3$ Hz)	6.23 (1H, *br d*, $J = 10.1$ Hz)	6.22 (1H, *br d*, $J = 10.1$ Hz)	6.08 (1H, *br d*, $J = 10.1$ Hz)	6.01 (1H, *br d*, $J = 10.1$ Hz)	6.03 (1H, *br d*, $J = 9.9$ Hz)	6.03 (1H, *br d*, $J = 10.1$ Hz),
3	3.98 (1H, *m*)	4.08 (1H, *m*)	4.30 (1H, *m*)	4.27 (1H, *br d*, $J = 6.0$ Hz)	4.20 (1H, *m*)	4.00 (1H, *br m*)	4.04 (1H, *m*)	4.10 (1H, *br s*)
4	2.63 (1H, *dd*, $J = 11.7, 5.0$ Hz) 1.63 (1H, *dd*, $J = 11.7, 10.1$ Hz)	2.88 (1H, *dd*, $J = 10.9, 5.5$ Hz) 1.57 (1H, *dd*, $J = 11.8, 10.0$ Hz)	3.04 (1H, *dd*, $J = 11.5, 10.5$ Hz) 2.16 (1H, *dd*, $J = 11.7, 5.9$ Hz)	2.92 (1H, *dd*, $J = 11.4, 10.4$ Hz) 2.09 (1H, *dd*, $J = 11.6, 5.8$ Hz)	3.18 (1H, *dd*, $J = 11.4, 10.6$ Hz) 1.89 (1H, *dd*, $J = 11.6, 5.0$ Hz)	2.44 (1H, *dd*, $J = 11.4, 5.6$ Hz) 1.83 (1H, *dd*, $J = 11.6, 11.2$ Hz)	2.42 (1H, *dd*, $J = 11.5, 5.4$ Hz) 1.86 (1H, *dd*, $J = 11.1, 10.7$ Hz)	1.82 (1H, *dd*, $J = 11.0, 10.9$ Hz) 2.46 (1H, *dd*, $J = 11.5, 5.6$ Hz)
7	5.97 (1H, *br s*)	5.98 (1H, *br s*)	5.85 (1H, *br s*)	5.81 (1H, *br s*)	5.69 (1H, *br s*)	5.74 (1H, *br s*)	5.80 (1H, *br s*)	5.73 (1H, *br s*)
8	–	–	5.26 (1H, *br d*, $J = 15.9$ Hz) 4.46 (1H, *dd*, $J = 15.9, 3.1$ Hz)	5.12 (1H, *br d*, $J = 15.6$ Hz) 4.02 (1H, *br d*, $J = 15.6$ Hz)	5.03 (1H, *br d*, $J = 15.2$ Hz) 4.12 (1H, *dd*, $J = 15.3, 3.3$ Hz)	4.01 (1H, *br d*, $J = 14.9$ Hz) 3.79 (1H, *dd*, $J = 14.8, 3.1$ Hz)	3.93 (1H, *br d*, $J = 15.4$ Hz) 3.85 (1H, *dd*, $J = 15.1, 2.1$ Hz)	4.04 (1H, *br d*, $J = 15.0$ Hz) 3.83 (1H, *dd*, $J = 15.3, 3.0$ Hz)
10	4.29 (1H, *dd*, $J = 14.2, 2.9$ Hz) 3.60 (1H, *dd*, $J = 14.3, 4.3$ Hz)	4.27 (1H, *dd*, $J = 13.9, 3.4$ Hz) 3.62 (1H, *dd*, $J = 13.9, 3.8$ Hz)	4.26 (1H, *br d*, $J = 13.7$ Hz) 4.07 (1H, *dd*, $J = 13.7, 3.8$ Hz)	4.03 (1H, *br d*, $J = 15.4$ Hz) 3.81 (1H, *dd*, $J = 15.4, 3.1$ Hz)	4.13 (1H, *br d*, $J = 14.8$ Hz) 3.68 (1H, *dd*, $J = 14.8, 4.5$ Hz)	3.25 (1H, *dd*, $J = 14.4, 3.0$ Hz) 3.46 (1H, *dd*, $J = 14.3, 4.1$ Hz)	3.63 (1H, *dd*, $J = 13.9, 4.1$ Hz) 3.15 (1H, *dd*, $J = 14.1, 3.4$ Hz)	3.44 (1H, *dd*, $J = 14.6, 4.1$ Hz) 2.29 (1H, *dd*, $J = 14.6, 2.8$ Hz)
11	4.77 (1H, *br s*)	4.51 (1H, *t*, $J = 3.8$ Hz)	5.11 (1H, *br d*, $J = 3.8$ Hz)	4.52 (1H, *d*, $J = 3.6$ Hz)	4.40 (1H, *d*, $J = 3.6$ Hz)	4.14 (1H, *dd*, $J = 3.4, 3.5$ Hz)	4.76 (1H, *t*, $J = 3.8$ Hz)	4.14 (1H, *dd*, $J = 3.2, 3.6$ Hz)
14	6.84 (1H, *s*)	6.99 (1H, *s*)	7.15 (1H, *s*)	6.84 (1H, *s*)	6.74 (1H, *s*)	6.99 (1H, *s*)	6.86 (1H, *s*)	6.85 (1H, *s*)
17	6.95 (1H, *s*)	7.02 (1H, *s*)	6.74 (1H, *s*)	6.72 (1H, *s*)	6.64 (1H, *s*)	6.89 (1H, *s*)	7.01 (1H, *s*)	6.91 (1H, *s*)
OCH$_3$	3.82 (3H, *s*) 3.71 (3H, *s*) 3.30 (3H, *s*)	3.82 (3H, *s*) 3.73 (3H, *s*) 3.45 (3H, *s*) 3.35 (3H, *s*)	3.85 (3H, *s*) 3.72 (3H, *s*) 3.36 (3H, *s*)	3.75 (3H, *s*) 3.57 (3H, *s*) 3.39 (1H, *s*)	3.82 (3H, *s*) 3.68 (3H, *s*) 3.52 (3H, *s*) 3.28 (3H, *s*)	3.79 (3H, *s*) 3.57 (3H, *s*) 3.35 (3H, *s*)	3.92 (3H, *s*) 3.79 (3H, *s*) 3.33 (3H, *s*)	3.91 (3H, *s*) 3.77 (3H, *s*) 3.58 (3H, *s*) 3.34 (3H, *s*)

of the C-11 methylene moiety by an oxymethine group. In the ^{13}C NMR, this moiety is in this case represented by the high field shifted signal at δ 64.3 and 75.0 (Table 1) for compounds **3** and **4**, respectively. This was confirmed from the ^{1}H NMR, which showed signals for these moieties appearing down field at δ 5.11 and 4.52 ppm, respectively. Compounds **3** and **4** had the same R_f and could only be resolved on TLC after seven ($\times 7$) developments. The only convincing differences between the two were seen in the chemical shifts of C-11 in their ^{13}C NMR spectra and their specific rotation values which were observed at +75 and +25, respectively for the two compounds. Compounds **3** and **4** thus differed only in the stereochemistry at C-11 and are thus epimers. The C-11 resonance is very diagnostic in differentiating between the α- and β-epimers (Table 1) in that the α-epimer this carbon will always resonate upfield (δ_C 60–66) while in the β-epimer it resonates slightly downfield (67–76). This carbon resonated at δ_C 64.3 and 74.5 for compounds **3** and **4**, respectively. The data obtained for compound **3** (Table 1) thus agreed with those of the compound reported as (+) erythrartine *N*-oxide whose C-11 hydroxyl group was erroneously assigned the β-configuration. A close examination of the data obtained for compound **3** however enabled placement of the hydroxyl group as having α- rather than the β-configuration (Table 1). Compound **3** was thus assigned the structure (+)-11α-hydroxyerysotrine *N*-oxide (**3**), while compound **4** was identified as (+)-11β-hydroxyerysotrine *N*-oxide.

Compounds **8** (isolated from the pods) and **9** (isolated from flowers) were both obtained as yellowish crystals both of molecular formulae $C_{19}H_{23}NO_4$, as deduced from the EI-MS data, which both showed molecular ion peaks at *m*/*z* 329.1. This was consistent with ^{13}C NMR results, which revealed the presence of 19 non-equivalent carbons. Further, the spectroscopic data obtained for these compound were similar to those of erysotrine (Amer et al., 1991a,b), already reported from a number of *Erythrina* species, except for the following differences: In both compounds **8** and **9**, the C-11 methylene moiety is replaced by an oxymethine group. The C-11 carbon signals for these epimers resonated at δ 73.9 and 64.9 ppm in the ^{13}C NMR while the attached protons resonated at δ 4.14 (1H, *dd*, $J = 3.4, 3.5$ Hz) and 4.76 (1H, *t*, $J = 3.8$), respectively, in the ^{1}H NMR spectra. In the ^{13}C NMR, the signals for C-10 for these compounds appeared at δ 46.2 and 51.3 ppm, respectively. Compounds **8** and **9** (just like **3** and **4**) thus differed only in stereochemistry at C-11 with compound **8** being the β-epimer while compound **9** is the α-epimer. Using these data (Table 1) compound **8** was assigned as (+)-11β-hydroxyerysotrine while **9** (previously thought to be the β-isomer) was assigned as (+)-11α-hydroxyerysotrine. (+)-Erythristemine, **14**, has been reported previously both in *E. lysistemon* and *Erythrina abyssinica* (Amer et al., 1991a) but only the ^{1}H NMR data was reported. In this paper we report for the first time the complete assignment of the ^{1}H- and ^{13}C NMR chemical shift using 2D NMR (COSY, HMQC and HMBC) spectroscopic techniques. These results enabled us to confirm the ^{1}H NMR ^{13}C NMR chemical attributions of **14** as previously reported in literature (Amer et al., 1991a). It is note worthy to mention that unlike the other erythrinaline alkaloids which seemed to be confined to flowers, pods and seeds, compound **14** was also found in the leaves, twigs and stem wood.

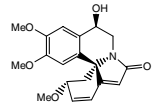
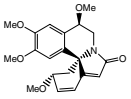
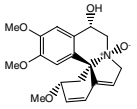
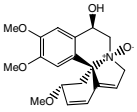
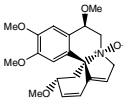
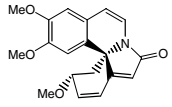
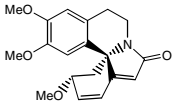
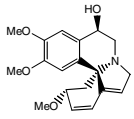
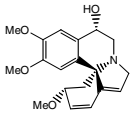
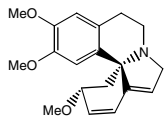
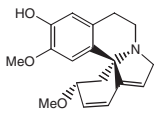
The current study has revealed the existence of both compounds **1** and **6** together in nature. Compound **1** may have been converted to **6** by dehydration, giving an olefinic moiety at C-10/C-11, the latter being a more stable structure considering the extended conjugation attained. Compound **6** has previously been isolated from *Erythrina arborescens* and was thought to be an artifact (Amer et al., 1991a). In our current finding, however, we have isolated both compounds **1** and **6**, and there was no evidence of the former converting to the latter even after leaving the former in solution for several days. Given this observation, the dehydration of **1** to give **6** may only be possible under enzymatic aid in the plant and thus **6**, by this reasoning, may well be a true natural product. The isolation of **2**, a 11-methoxy derivative of **1** also seems to corroborate the fact that **1** is fairly stable and not prone to dehydration.



1 **6**

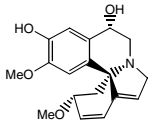
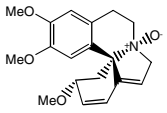
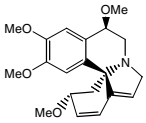
The chloroform and methanol extracts of the pods were also examined and found to have some alkaloids common to those found in the flowers (Table 3). A comparison of the current results with those reported (Amer et al., 1991a,b) from seeds showed the seeds to contain compounds **9**–**14** (Table 3). Other erythrinaline alkaloids reported only in the seeds are (+)-erythravine, (+)-erythraline, (+)-8-oxoerythraline, (+)-11β-methoxyerythraline, (+)-8-oxo-11β-methoxyerythraline, (+)-glucoerysodine, (+)-11β-methoxyglucoerysodine, (+)-11β-hydroxyerysodine, (+)-11β-methoxyerysodine, (+)-rhamnoerysodine, (+)-erysovine, (+)-11β-methoxyglucoerysovine, (+)-11-hydroxyerysovine and (+)-11-methoxyerysovine (Amer et al., 1991a,b; NAPRALERT, 2003) whereby the stereochemistry at C-11 for the last two compounds could not be specified due to insufficient spectral data and other forms of chemical evidence. There was no indication of these in either the flowers or pods in our study. It appears, from this study, that free alkaloids are most abundant in the flowers while the seeds have, in addition, some glycosylated alkaloids.

Table 3
Compounds isolated from flowers and pods of *Erythrina lysistemon* and their antioxidant activity

Compound	Flowers	Pods	Seeds	DPPH assays		
				On TLC (μg)	IC_{50} (μg/ml, 30 min)	IC_{50} (μg/ml, 4 h)
1  N	√	√	×	100	>300	>300
2  N	√	×	×	100	>500	>500
3  MI	√	×	×	100	>300	>300
4  N	√	√	×	50	>500	>500
5 	√	×	×	100	>300	>300
6 	√	×	×	100	NT	NT
7 	√	×	×	50	>500	>500
8  N	√	√	×	50	280	280
9  MI	√	×	√	50	210	210
10 	√	×	√	50	>500	>500
11 	√	×	√	1	150	90

(*continued on next page*)

Table 3 (*continued*)

Compound	Flowers	Pods	Seeds	DPPH assays		
				On TLC (μg)	IC_{50} (μg/ml, 30 min)	IC_{50} (μg/ml, 4 h)
12 	×	√	√	0.1	200	160
13 	√	×	√	50	700	700
14 	√	√	√	10	>500	>500

Standards: Ascorbic acid $IC_{50} = 16$ μg/ml (0.5 μg, TLC) and gallic acid $IC_{50} = 8$ μg/ml (<0.5 μg, TLC); MI, earlier misidentified; N, new; NT, not tested; √, compound present; ×, compound absent.

2.1. DPPH radical scavenging activity

To evaluate the potential antioxidant activity, DPPH was used to determine the radical scavenging ability of the compounds isolated. On interaction with DPPH, antioxidants either transfer electrons or hydrogen atoms to DPPH and thus neutralize the free radical character (Naik et al., 2003). DPPH is a stable free radical and any molecule that can donate an electron or hydrogen to it reacts with it thus bleaching its absorption. In investigating the radical scavenging ability of the alkaloids isolated here, a number of them showed radical scavenging ability on the TLC assay (Table 3) but only compounds **11** and **12** were active in the spectrophotometric assay with IC_{50} values of 150 and 200 μg/ml after 30 min and 90 and 170 μg/ml after 4 h, respectively. The fact that the IC_{50} values change after 4 h seem to suggest that these compounds react slowly in the assay and therefore maybe classified as slow kinetic antioxidants (Brand-Williams et al., 1995).

Though the crude $CHCl_3$/MeOH (1:1) extract showed some good brine shrimp toxicity (LC_{50} 23 ppm), surprisingly, none of the isolated compounds exhibited activity below 100 ppm. This finding seem to point to the fact that either these alkaloids act synergistically as a group (not very likely) or that toxicity is due to something else other than alkaloids which we missed out.

3. Experimental

3.1. General

Optical rotation $[\alpha]_D$: 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, HMBC, HMQC: Bruker Avance DPX 300 Spectrometer using standard pulse sequences and referenced to residual solvent signal. EI-MS: Finnigan MAT SSQ 7000 Single Quadrupole Instrument at 70 eV. CC: Silica gel 60 (0.040–0.060 mm, Merck). Preparative and analytical TLC: Silica gel 60 $PF_{254+366}$ (Merck). Visualization of chromatograms (UV at λ 254 and 366 nm) and vanillin–sulphuric acid spray.

3.2. Plant material

Flowers of *E. lysistemon* Hutch. were collected in July 2001 in Gaborone (on campus), Botswana and its voucher specimen (No. EL 0701) is preserved in the University Herbarium, Department of Biological Sciences, University of Botswana.

3.3. Extraction and isolation

The flowers (ca. 2 kg) were crushed while still wet using a blender and extracted for 24 h with 1:1 $CHCl_3$/MeOH mixture (2 L) at room temperature three times. The extract was concentrated in vacuo to give 65 g of a brown residue. This crude extract was suspended in water and partitioned successively between chloroform and *N*-butanol. The chloroform soluble fraction (25 g) was chromomatographed on silica gel (300 g) and eluted using *N*-hexane/$CHCl_3$, $CHCl_3$ and then $CHCl_3$/MeOH mixtures with increasing polarities to afford 10 major fractions (frs A–J) based on TLC analysis. Fraction D was subjected to CC (hex/$CHCl_3$ in increasing polarity) to give four major fractions D_1–D_4 (monitored on

TLC). D_2 was further subjected to multiple development (×3) on prep. TLC ($CHCl_3$) resulting in the isolation of (+)-11β-methoxyerysotramidine **2** (25 mg). Fraction E was also subjected to CC with the column on a step-wise elution with $CHCl_3$ and then $CHCl_3$/MeOH resulting in fractions E_1–E_5. Fraction E_3 was then subjected to prep. TLC (×2 developments, 1% MeOH in $CHCl_3$) and further purified using Sephadex LH-20 to obtain (+)-11β-hydroxyerysotramindine **1** (10 mg). Fraction G was resolved on a silica gel column eluting with $CHCl_3$ followed by $CHCl_3$/MeOH in increasing polarity to obtain six major fractions, G_1–G_6. G_6, eluted from the column using 6% MeOH in $CHCl_3$, only showed two spots after multiple developments on TLC. Compounds **3** (10 mg) and **4** (15 mg) were resolved from this fraction after seven (×7) developments on prep. TLC. G_2 was further subjected to CC (eluting with 2% MeOH in $CHCl_3$) followed by purification on Sephadex LH-20 and fractional crystallization yielded **5** (30 mg). Similar treatment of G_1–G_5 gave **6** (5 mg), **7** (10 mg), **8** (5 mg), **9** (31 mg), 1**0** (16 mg), **11** (12 mg), **13** (21 mg) and **14** (102 mg).

Air-dried pods were ground to coarse powder (3 kg) and subsequently extracted sequentially with chloroform and methanol, which upon solvent evaporation gave in each case 16 and 42 g, respectively, of dark green material. The methanol extract was subjected to flash chromatography on silica gel using $CHCl_3$, then $CHCl_3$/MeOH mixtures with increasing polarity, resulting in fractions A–K. Fraction F (770 mg) was then subjected to gradient elution chromatography on silica gel column using $CHCl_3$ then $CHCl_3$/MeOH mixtures to give fractions F_1–F_{10}. Subjecting fraction F_2 to prep. TLC followed by cleaning using Sephadex LH-20 afforded compound **8** (67 mg). Similar treatment of F_1, F_3–F_{10} gave **1** (3 mg), **4** (8 mg), **12** (7 mg) and **14** (20 mg). The leaf, twig and stem wood materials were treated similarly to yield 5, 18 and 18 mg, respectively, of compound **14**. Identification of these compounds was done by comparison of physical and spectral data with those published in literature (Amer et al., 1991a,b; NAPRALERT, 2003).

3.4. DPPH assay

Reduction of DPPH (2,2-diphenyl-1-picrylhydrazyl or 2,2-diphenyl-1-(2,4,6-trinitrophenyl)-hydrazyl) radical [molecular formula $C_{18}H_{12}N_5O_6$, molecular weight 394]. TLC autographic assay: after developing and drying, TLC plates (with amounts of sample ranging from 0.1 to 100 μg) were sprayed with 0.2% (2 mg/ml) of DPPH solution in methanol. The plates were examined half an hour after spraying. Active compounds appeared as yellow spots against a purple background.

One millilitre of a 500 μM (0.2 mg/ml) DPPH in methanol was mixed with equal volumes of test compounds at various concentrations, mixed well and kept in the dark for 30 min. The absorbance at 517 nm was monitored in the presence of different concentrations of the samples. Blank experiment was also carried out to determine the absorbance of DPPH before interacting with the compounds. The amount of sample in μg/ml at which the absorbance at 517 nm decreases to half its initial value is used as the IC_{50} value of the compound.

3.5. Physical and spectroscopic data

3.5.1. (+)-11β-Hydroxyerysotramidine (*1*)

Yellowish solid, $[\alpha]_D$+100 ($c = 0.14$, MeOH); UV (MeOH) $\lambda_{max}(\log \varepsilon)$ 322 (2.49), 257 (3.52), 219 (2.98) nm; ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Tables 1 and 2. EI-MS *m/z* (rel. int.) 343 $[M]^+$(100), 328 $[M - CH_3]$ (51), 310 (72), 301 (10), 282 (15), 268 (10), 254 (28), 223 (7).

3.5.2. (+)-11β-Methoxyerysotramidine (*2*)

Brownish solid, $[\alpha]_D$+60 ($c = 0.11$, MeOH); UV (MeOH) $\lambda_{max}(\log \varepsilon)$ 325 (1.58), 238 (3.28), 220 (2.96) nm; ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Tables 1 and 2. EI-MS *m/z* (rel int.) 357 $[M]^+$(91), 342 $[M - CH_3]$ (82), 326 $[M - OCH_3]$ (100), 311 (92), 296 (36), 287 (24), 269 (30), 254 (35), 225 (10), 208 (8), 181 (40).

3.5.3. (+)-11α-Hydroxyerysotrine N-oxide (*3*)

Yellowish oily material, $[\alpha]_D$+100 ($c = 0.04$, MeOH); UV (MeOH) $\lambda_{max}(\log \varepsilon)$ 338 (2.42), 240 (3.32), 220 (2.82) nm; ^{1}H NMR (300 MHz, acetone-d_6) and ^{13}C NMR (75 MHz, acetone-d_6), see Tables 1 and 2. EI-MS *m/z* (rel int.) 345 $[M]^+$(62), 329 [M − O] (100), 313 (48), 296 (18), 282 (13), 266 (9).

3.5.4. (+)-11β-Hydroxyerysotrine N-oxide [(erythrartine N-oxide)] (*4*)

Light brown oily substance, $[\alpha]_D$+25 ($c = 0.04$, MeOH); UV (MeOH) $\lambda_{max}(\log \varepsilon)$ 327 (2.31), 260 (3.61), 219 (2.90) nm; ^{1}H NMR (300 MHz, acetone-d_6) and ^{13}C NMR (75 MHz, acetone-d_6), *see Tables 1 and 2. EI-MS m/z* (rel int.) 345 $[M]^+$(73), 329 [M − O] (30), 327 (100), 313 (14), 296 (13), 278 (4), 269 (2).

3.5.5. (+)-11β-Hydroxyerysotrine (*8*)

Yellowish crystals, EI-MS *m/z* (rel. int.): 329 $[M]^+$(75), 314 (15), 298 (100), 282 (24), 266 (32), 250 (10), 234 (6), 222 (5), 213 (3); ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Tables 1 and 2.

3.5.6. (+)-Erythristemine (*14*)

Light brown paste, $[\alpha]_D$ +176.9 (MeOH) EI-MS *m/z* (rel. int.): 343 $[M]^+$ (100), 312 (20), 280 (25), 265 (35), 249 (32), 234 (5): ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Tables 1 and 2.

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References

Allen, O.N., Allen, E.K, 1981. The Leguminosae: A Source Book of Characteristic Uses and Nodulation. Macmillan, USA, p. 275.

Amer, M.E., Shamma, M., Freyer, A.J., 1991a. The tetracyclic *Erythrina* alkaloids. J. Nat. Prod. 54, 329–363.

Amer, M.E., El-Masry, S., Shamma, M., Freyer, A.J., 1991b. Three novel glucodienoid alkaloids from *Erythrina lysistemon*. J. Nat. Prod., 54,161–166.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. Lebensm.-Wiss.-Technol. 28, 25–30.

El-Masry, S., Amer, M.E., Abdel-Kader, M.S., Zaatout, H.H., 2002. Prenylated flavonoids of *Erythrina lysistemon* grown in Egypt. Phytochemistry 60, 783–787.

Fabian, A., Germishhuizen, G., 1997. Wild flowers of Northern South Africa. Fernwood Press, Vlaeberg, pp. 160–200, 400–408.

McKee, T.C., Bokesch, H.R., McCormick, J.L., Rashid, M.A., Spielvogel, D., Gustafson, K.R., Alavanja, M.M., Cardellina II, J.H., Boyd, M.R., 1997. Isolation and characterization of new anti-HIV and cytotoxic leads from plants, marine and microbial organisms. J. Nat. Prod. 60, 431–438.

Naik, G.H., Priyadarsini, K.I., Satav, J.G., Banavalikar, M.M., Sohoni, D.P., Biyani, M.K., Mohan, H., 2003. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. Phytochemistry 63, 97–104.

NAPRALERT (Natural Products Alert) Internet database, October 20, 2003. Ethnopharmacology, biological activity and phytochemical information on genus *Erythrina*.

Obiero, G.O., 2001. Chemical and antibacterial constituents of *Erythrina lysistemon*. M.Sc. Thesis, University of Botswana, Gaborone, pp. 30–50.

Venter, F., Venter, J.-A., 1996. Making the Most of Indigenous Trees. Briza Publications, Pretoria, p. 232.