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Alkaloids from the flower of *Erythrina arborescens*†

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Phytochemical investigations on the flower of *Erythrina arborescens* resulted in the isolation of eight new *Erythrina* alkaloid, erytharborines A–H (**1–8**), together with 17 known alkaloids. Erytharborines A/B (**1–2**) and C (**3**) possessed an 2*H*-imidazole ring and a unique oxime moiety, respectively. The structures were elucidated on the basis of UV, IR, mass spectrometry and NMR spectroscopic data.

Introduction

The *Erythrina* and *Homoerythrina*-type alkaloids, derived from two tyrosine units *via* oxidative coupling and intramolecular rearrangement, consist of more than 200 alkaloids from *Erythrina* and *Cephalotaxus* genus.[1,2] The erythrinan alkaloids are ubiquitous compounds in the *Erythrina* genus of family *Leguminosae*. Special attention has been received in this field mainly by their curare-like neuro muscular blocking activities,[3] anxiolytic-like activity,[4] induced sleep,[5] anticonvulsant activity,[6] anticataract[7] and antifeedant[8] activity *etc.* Particularly noteworthy, the star molecule, dihydro-β-erythroidine, was used as tool to characterize neuronal nicotinic acetyl-choline receptors.[9] Thus, pharmaceutical chemists paid much more attention to this type natural products. The erythrinan alkaloids possessed 6/5/6/6 spirocycle systems with a stable 5*S*-chiral center, seemingly exhibiting a not so diverse and fascinating molecular architecture. Nevertheless, the spirocyclic and aromatic skeleton in erythrinan alkaloids became challenging polycyclic molecular architectures.[10–12] Generally speaking, skeleton rearrangement served as the main pathway to structural diversity of natural products. Analogously, besides aromatic erythrinan alkaloid (erysotramidine[13]), this class compound also included nonaromatic alkaloid, *e.g.* six-membered lactone (β-erythroidine[14]) and pyridine ring D (erymelanthine[15]). Both molecules attracted many interests in total synthesis.[1,16,17] Under considerable efforts of our research group devoted to the phytochemical investigations on *Erythrina* species, several novel dimeric and trimeric erythrinan alkaloids some of which showed cytotoxicity were obtained.[18,19] As part of an ongoing research for structural newly erythrinan alkaloids, phytochemical investigation of the flowers of *Erythrina arborescens* Roxb. led to eight new alkaloids erytharborines A–H (**1–8**) (Fig. 1) together with seventeen known alkaloids. Their isolation and structure elucidation were described in this study.

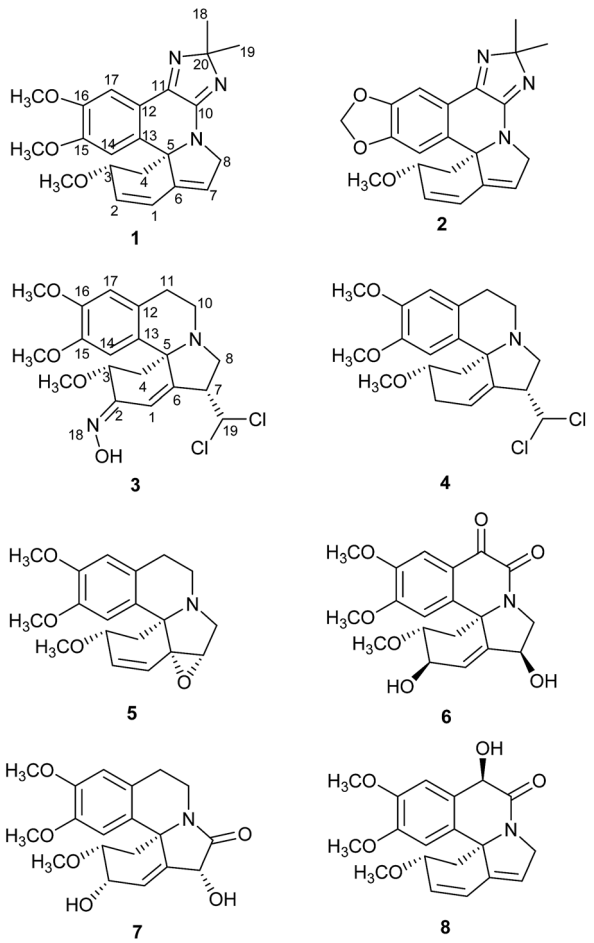

Fig. 1 Structures of erytharborines A–H (**1–8**).

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Results and discussion

The alkaloid fraction of *E. arborescens* was separated to yield a total of 25 compounds by a combination of chromatographic procedures as described in the Experimental section. All compounds might be alkaloids since they showed positive response with Dragendorff's reagent on TLC.

The UV absorptions (202, 227, 289 and 322 nm) and IR spectrum (1710, 1629, 1479 cm^{-1}) of erytharborine A (**1**) indicated a good conjugated system. Presence of the typical conjugate olefin signals (δ_H 6.81, 6.04, 5.96), two aromatic singlet protons (δ_H 7.57 and 7.27) and three methoxyl groups (δ_H 3.90, 3.81 and 3.20) in the ^1H NMR spectrum of **1**, displayed the untapped A, B and D-rings of conjugated dienoid type erythrinan alkaloids. Two characteristic methylenes at δ_C 48.7 and 56.9 in the ^{13}C NMR spectrum together with their HMBC correlations assigned themselves to C-4 and C-8, respectively. The untapped A, B and D-rings of **1** was further supported by its key correlations observed in the HMBC spectrum, δ_H 6.81 (H-1)/δ_C 76.8 (C-3) and 71.5 (C-5), δ_H 6.04 (H-2)/δ_C 48.7 (C-4), 140.4 (C-6), δ_H 5.96 (H-7)/δ_C 125.7 (C-1) and 71.5 (C-5), δ_H 7.27 (H-14)/δ_C 71.5 (C-5), 119.6 (C-12) and 149.9 (C-16), δ_H 7.57 (H-17)/δ_C 136.9 (C-13) and 152.4 (C-15) (Fig. 2). Its molecular formula $C_{22}H_{25}N_3O_3$ was deduced from HRESIMS at $m/z = 380.1961$ $[M + H]^+$ (calcd. 380.1969), with three more carbons including two methyl groups (δ_C 25.0, 25.9) than general *Erythrina* alkaloid. In the HMBC spectrum, the correlations between H-17 and δ_C 155.4 (s) attributing the latter signal to C-11. Likewise, the correlations between H-8 (δ_H 4.56, 4.25) with δ_C 157.4 (s) attributing the latter signal to C-10. The HMBC correlations of δ_H 1.46 (3H) and 1.39 (3H) with δ_C 104.3 (s) established the linkage of the three carbons. Based on the molecular formula, 2*H*-imidazole ring was necessary in consideration of remainder unsaturation degrees of **1** (Fig. 2). In the ROESY spectrum, the NOE correlation of H-3/H-14 suggested H-3 was in β-orientation.

Erytharborine B (**2**) was obtained as pale yellow amorphous powder with similar UV and IR absorption to **1**. Its molecular formula was confirmed to be $C_{21}H_{21}N_3O_3$ by HRESIMS at $m/z = 364.1658$ $[M + H]^+$ (calcd. 364.1656), with 14 daltons more than **1**. Comparing their closely resembled ^1H and ^{13}C NMR data value (Table 1), compound **2** must possess a methylenedioxyl group (δ_H 6.12 and 6.09) at C-15 and C-16 in place with the two methoxyl groups (δ_H 3.90 and 3.81) in **1**.

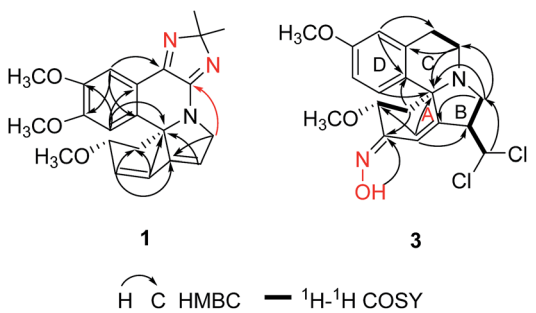

Fig. 2 Key HMBC and ^1H–^1H COSY correlations of **1** and **3**.

The UV absorption of erytharborine C (**3**) at 204 and 289 nm indicated a tetrahydroisoquinoline chromophore.[20] Meanwhile, its IR absorption bands at 3414 and 1611, 1513, and 1458 cm^{-1} resulted from the hydroxyls and aromatic rings, which was consistent with the characteristic of *Erythrina* alkaloid. Its molecular formula was determined to be $C_{20}H_{24}N_2O_4Cl_2$ based on HRESIMS at $m/z = 427.1197$ $[M + H]^+$, indicating nine degrees of unsaturation. The isotope peaks showed in the positive ESI-MS confirmed the presence of two chlorine atoms. The ^1H-, ^{13}C NMR and HSQC data for **3** indicated the presence of four methylenes, three methoxyls, three sp^3 and three sp^2 methines, one sp^3 and six sp^2 quaternary carbons. Above data further suggested **3** was similar to erthratidinone[21] except for an additional carbon and nitrogen, and two chlorine atoms. In the HMBC spectrum, correlations from δ_H 6.71 (H-17) to δ_C 20.6 (C-11), δ_C 126.3 (C-13), and δ_C 147.8 (C-15), and from δ_H 6.28 (H-14) to δ_C 63.7 (C-5), δ_C 125.5 (C-12), and δ_C 146.1 (C-16) suggested D-ring was not changed.[20] Its ^1H–^1H COSY correlations of H-11 (δ_H 2.98 and 2.49) to H-10 (δ_H 3.09 and 3.30), together with the correlation of H-10 with C-5 in the HMBC spectrum indicated C-ring was untapped. The coupling –CH_2 (δ_H 2.94 and 3.18) and –CH (δ_H 3.66) correlated with C-6 in the HMBC spectrum, respectively, also assigned them to H-8 and H-7 and suggested ring-B was substituted. The methine δ_H 5.86 was attributed to newly CH-19 based on its ^1H–^1H COSY correlation with H-7, which was supported by the HMBC correlations from H-19 to C-8. Downfield proton and carbon signal of CH-19 meant linkage with two Cl atoms, also consideration of its molecular formula. Finally, singlet signal H-1 (δ_H 7.07) showed HMBC correlations with C-7 and C-5 suggested a double bond at C-1/6. Correlations of H-3 (δ_H 4.14)/H-4 (δ_H 2.71 and 1.68) in the ^1H–^1H COSY spectrum together with the HMBC correlations between H-4 with δ_C 151.6 assigned the signal to C-2. The remainder of a nitrogen atom and degree of unsaturation suggested there should be an *E*-oxime moiety as shown in Fig. 2,[22] which was supported by HMBC correlation of δ_H 11.20 (OH) with C-2.

The molecular formula $C_{20}H_{25}NO_3Cl_2$ of alkaloid (**4**) was established by HRESIMS ($[M + H]^+$ at m/z 398.1281) and was consisted with the ^{13}C NMR spectrum, which revealed 20 carbonic resonance signal. The 1D NMR spectroscopic data of compound **4** were similar to those of compound **3** except for the following differentiations: in the ^1H NMR spectrum, the signal displayed at δ_H 11.20 in **3** which was assigned to the active hydrogen in the oxime moiety was disappeared in compound **4**. Correspondingly, the quaternary carbon signals at δ_C 151.6 (C-2) in compound **3** was replaced with a methylene (δ_C 33.1) in compound **4**. Thus, compound **4** might be an analogue of **3** without the oxime moiety. The HMBC correlations of δ_H 2.05 (H-2)/δ_C 125.4 (C-1), δ_C 74.1 (C-3) and δ_C 140.5 (C-6) together with the HSQC data demonstrated directly that the methylene did belong to C-2. Relative configuration of H-3 in **3** and **4** was deduced as β from the coupling constants ($J_{3,4eq.} = 5.0$ Hz, $J_{3,4ax} = 11.0$ Hz) in the ^1H NMR spectrum.[23] This presumption was confirmed by the obvious NOE correlations of H-3/H-14. Likewise, the correlations of H-7/H-17 in the ROESY spectrum showed H-7 in **3** and **4** was β-oriented, too. The oxime of C_2/N_{18} in **3** was determined as *E via* NOE between OH and H-1.

Table 1 ^{13}C NMR spectroscopic data for **1–8** in acetone-d_6 (δ in ppm)

Entry	δ_C (**1**)	δ_C (**2**)	δ_C (**3**)[a]	δ_C (**4**)[a]	δ_C (**5**)[b]	δ_C (**6**)[a]	δ_C (**7**)[a]	δ_C (**8**)[b]
1	125.7 d	125.6 d	114.5 d	125.4 d	128.6 d	125.4 d	123.8 d	124.8 d
2	132.6 d	132.6 d	151.6 s	33.1 t	136.9 d	72.5 d	62.8 d	132.8 d
3	76.8 d	76.6 d	73.4 d	74.1 d	76.5 d	82.0 d	76.9 d	77.1 d
4	48.7 t	48.6 t	42.9 t	43.0 d	46.5 t	49.3 t	35.6 t	40.5 t
5	71.5 s	71.2 s	63.7 s	64.5 s	66.1 s	65.2 s	62.3 s	72.3 s
6	140.4 s	140.1 s	150.1 s	140.5 s	71.2 s	144.8 s	140.7 s	139.7 s
7	120.9 d	121.4 d	51.3 d	53.5 d	64.6 d	69.2 d	70.7 d	120.8 d
8	56.9 t	56.6 t	49.9 t	53.2 t	56.4 t	51.2 t	172.6 s	54.7 t
10	157.5 s	157.1 s	39.8 t	40.8 t	51.6 t	159.4 s	35.8 t	173.8 s
11	155.4 s	155.6 s	20.6 t	21.7 t	29.4 t	180.7 s	25.9 t	68.3 d
12	119.6 s	121.4 s	125.5 s	130.2 s	130.9 s	125.4 s	126.8 s	129.4 s
13	136.9 s	138.7 s	126.3 s	126.4 s	131.2 s	140.0 s	131.9 s	131.0 s
14	108.2 d	105.3 d	110.9 d	112.7 d	110.1 d	109.4 d	110.3 d	108.4 d
15	152.4 s	151.5 s	147.8 s	149.5 s	148.0 s	154.2 s	147.9 s	148.7 s
16	149.9 s	148.5 s	146.1 s	147.7 s	148.8 s	150.3 s	149.9 s	149.9 s
17	109.9 d	107.2 d	112.7 d	113.6 d	112.7 d	110.5 d	114.3 d	109.3 d
18	25.0 q	25.7 q						
19	25.9 q	26.9 q	74.3 d					
20	104.3 s	100.5 s						
3-OCH_3	56.2 q	56.6 q	58, 5 q	55.9 q	56.0 q	56.2 q	56.0 q	56.1 q
15-OCH_3	56.1 q		55.4 q	56.1 q	56.3 q	56.6 q	56.2 q	56.3 q
16-OCH_3	56.1 q		55, 3 q	56.1 q	56.4 q	56.6 q	56.3 q	56.3 q
OCH_2O		103.0 t						

[a] ^{13}C NMR recorded in 150 MHz. [b] ^{13}C NMR recorded in 125 MHz. Compound **3** was recorded in DMSO-d_6.

Erytharborine E (**5**) was isolated as an amorphous solid. Its molecular formula was deduced as $C_{19}H_{23}N_2O_5$ from the HRESIMS ($[M + H]^+$ at 330.1699) and ^{13}C NMR spectroscopic data, inferring nine degrees of unsaturation. In comparing with the ^{13}C NMR data of erysotrine,[13] compound **5** showed a oxygenated quaternary carbon (δ_C 71.2) and a oxygenated methine (δ_C 64.6) at up-field instead of olefinic signals of δ_C 143.4 (s, C-6) and δ_C 123.6 (d, C-7) of erysotrine, which suggested presence of an epoxide ring at C-6/7. The HMBC correlations of δ_H 2.87 (H-8)/δ_C 64.6 (C-7), δ_C 71.2 (C-6), δ_H 5.76 (H-1)/δ_C 64.6 (C-7) and δ_H 6.25 (H-2)/δ_C 71.2 (C-6) confirmed this conclusion. The epoxide was assigned as β-orientation on the base of molecule model.

Erytharborine F (**6**) was obtained as a white amorphous powder. Its molecular formula was determined to be $C_{19}H_{21}NO_7$ based on its HRESIMS at *m*/*z* 398.1212 ($[M + Na]^+$) and NMR spectra. The ^{1}H NMR spectra (Table 2) confirmed the presence of two aromatic singlet protons (δ_H 7.36 and 7.18), one olefinic proton (δ_H 6.18) and three methoxyls (δ_H 3.93, 3.90 and 3.20). Its ^{1}H- and ^{13}C NMR data resembled those of (+)-10,11-dioxoepierythratidine[24] with exception for an additional hydroxyl group, which was deduced from its molecular formula. Substitution of hydroxyl group at C-7 was supported by the HMBC correlations of δ_H 6.18 (H-1)/δ_C 69.2 (C-7), δ_H 4.31 (H-8)/δ_C 69.2 (C-7) and δ_H 4.98 (H-7)/δ_C 144.8 (C-6). The signals at δ_H 7.36 (H-17) showed correlation with the δ_C 180.7 (C-11) in the HMBC spectrum, while the signals at δ_H 4.31 (H-8) showed correlation with the δ_C 154.9, establishing dione at C-10/11. The hydroxyl groups at C-2 and C-7 were both β-oriented as deduced from the NOESY correlations of H-2/H-4$_{ax}$, H-8$_{ax}$/H-4$_{ax}$, H-7/H-8$_{ax}$.

Erytharborine G (**7**), a white amorphous powder, had the molecular formula $C_{19}H_{23}NO_6$ as deduced from its HRESIMS ($[M + Na]^+$ at *m*/*z* 384.1417) and NMR spectra. The pattern of ^{13}C NMR data for **7** were similar to those of **6** except that the former contained only one carbonyl group in low-field. In the HMBC spectrum, correlations of δ_H 6.81 (H-17)/δ_C 25.9 (C-11, t) and δ_H 3.00 (H-11)/δ_C 35.8 (C-10, t) indicated that the carbonyl group was neither located at C-10 nor at C-11. The HMBC correlation between δ_H 4.36 (H-7) and δ_C 172.6 (C=O) assigned the carbonyl group to C-8 position. Its ROESY spectrum gave correlations of H-3/H-14, H-2/H-14 and H-7/H-14, which demonstrated the relative configuration of H-2, H-3 and H-7 were β-oriented.

The molecular formula erytharborine H (**8**) was established as $C_{19}H_{21}NO_5$ based on the HRESIMS ($[M + Na]^+$ at 366.1310) and ^{13}C NMR spectroscopic data. The ^{1}H NMR spectrum showed the presence of two aromatic singlet protons (δ_H 7.23 and 7.02) and three conjugated olefinic protons (δ_H 6.75, 6.04, and 5.86), which were the characteristic signals to *Erythrina* alkaloid with a 1/2,6/7-diene system. When compared with 10-hydroxy-11-oxoerysotrine,[25] compound **8** showed great similarity in ^{1}H and ^{13}C NMR data. In the HMBC spectra, correlations between H-8 (δ_H 4.31) and δ_C 173.8 (C=O) attributed the carbonyl to C-10 position other than C-11. Likewise, the hydroxyl group was determined to be attached at C-11 by HMBC correlations of δ_H 7.23 (H-17) to δ_C 68.3 (C-11). Therefore, compound **8** was defined as 10-oxo-11-hydroxyerysotrine. On the basis of the ROESY experiment, a correlation of H-3/H-4 eq. and H-4 eq./H-11 assigned the 11-OH group as being β-oriented.

The positive optical rotation value of **1–8** suggested that they had same configuration at C-5.[24,26] As the main constituent, alkaloid **23** showed same optical rotation ($[\alpha]_D^{23}$ + 206 (c = 0.36, CH_3OH)) as previous reported erythrinine.[16] So **1–8** should possess identical 5s-configuration, and named as

Table 2 ^{1}H NMR spectroscopic data for **1–8** in acetone-d_6 (J in Hz)

Entry	δ_H (**1**)[a]	δ_H (**2**)[a]	δ_H (**3**)[a]	δ_H (**4**)[a]	δ_H (**5**)[b]	δ_H (**6**)[a]	δ_H (**7**)[a]	δ_H (**8**)[b]
1	6.81 (dd, 10.2, 2.4)	6.78 (dd, 10.3, 2.4)	7.07 (s)	5.97 (t, 3.7)	5.76 (brd, 10.4)	6.18 (d, 4.9)	6.20 (br, s)	6.75 (br, d, 10.3)
2	6.04 (d, 10.2)	6.01 (d, 10.2)		2.88 (overlap), 2.05 (overlap)	6.25 (brd, 10.4)	4.38 (dd, 4.9, 4.2)	4.60 (dd, 4.3, 3.2)	6.04 (d, 10.3)
3	3.76 (m)	3.72 (m)	4.14 (m)	3.83 (m)	3.79 (m)	3.41 (dd, 12.0, 5.0)	3.63 (dt, 11.9, 3.2)	3.72 (dd, 11.5, 5.3)
4	2.21 (dd, 11.3, 5.2), 1.95 (dd, 11.3, 10.2)	2.21 (dd, 11.3, 5.3), 1.95 (d, 11.3)	2.71 (dd, 11.0, 5.0) 1.68 (t, 11.0)	2.29 (dd, 11.0, 5.0), 1.44 (t, 11.0)	2.15 (dd, 12.6, 5.0), 1.94 (dd, 12.6, 10.0)	2.16 (t, 12.0), 2.08 (dd, 12.0, 5.0)	2.13 (dd, 11.9, 3.2), 1.94 (t, 11.9)	2.76 (dd, 11.5, 5.3), 1.86 (t, 11.5)
7	5.96 (d, 2.4)	5.97 (d, 2.8)	3.66 (dd, 7.0, 3.5)	3.27 (m)	3.61 (overlap)	4.69 (dd, 7.8, 6.0)	4.36 (d, 6.0)	5.86 (s)
8	4.56 (dd, 15.8, 2.4), 4.25 (d, 15.8)	4.55 (dd, 15.8, 2.8), 4.24 (d, 15.8)	2.94 (dd, 10.0, 3.5), 3.18 (dd, 10.0, 7.0)	3.20 (overlap), 2.60 (dd, 9.9, 6.7)	3.61 (overlap), 2.89 (d, 12.5)	4.31 (dd, 10.2, 7.8), 3.10 (t, 10.2)		4.31 (2H, br, s)
10			3.09 (m), 3.30 (overlap)	3.44 (m), 3.09 (m)	3.13 (m), 2.44 (m)		4.03 (m), 3.47 (m)	
11			2.98 (m), 2.49 (m)	3.01 (m), 2.54 (m)	2.73 (m), 2.60 (m)		3.02 (2H, overlap)	5.38 (s)
14	7.27 (s)	7.15 (s)	6.28 (s)	6.55 (s)	7.12 (s)	7.18 (s)	6.33 (s)	7.02 (s)
17	7.57 (s)	7.49 (s)	6.71 (s)	6.71 (s)	6.78 (s)	7.36 (s)	6.81 (s)	7.23 (s)
18	1.46 (3H, s)	1.45 (3H, s)						
19	1.39 (3H, s)	1.38 (3H, s)	5.86 (d, 6.0)					
3-OCH_3	3.20 (3H, s)	3.21 (3H, s)	3.27 (3H, s)	3.22 (s)	3.23 (3H, s)	3.20 (3H, s)	3.29 (3H, s)	3.24 (3H, s)
15-OCH_3	3.81 (3H, s)		3.65 (3H, s)	3.77 (s)	3.79 (3H, s)	3.93 (3H, s)	3.81 (3H, s)	3.84 (3H, s)
16-OCH_3	3.90 (3H, s)		3.75 (3H, s)	3.72 (s)	3.71 (3H, s)	3.90 (3H, s)	3.79 (3H, s)	3.72 (3H, s)
OCH_2O		6.12 (br, s), 6.09 (br, s)						
2-OH						4.84 (d, 4.2)	3.59 (d, 4.3)	
7-OH						4.98 (d, 6.0)	4.96 (d, 6.0)	
11/18-OH			11.20 (s)					4.43 (s)

[a] ^{1}H NMR recorded in 600 MHz. [b] ^{1}H NMR recorded in 400 MHz; compound **3** was recorded in DMSO-d_6.

erytharborines A-H, respectively. Additionally, all reported *Erythrina* and *Homoerythrina*-type alkaloids have this configuration so far.

The known alkaloids were identified as, erytharbine (**9**),[25] 8-oxoerthraline epoxide (**10**),[27] erythratidinone (**11**),[21] erythratine (**12**),[28] erysotramidine (**13**),[13] 10,11-dioxoerysotrine (**14**),[29] 11β-hydroxyerysotramidine (**15**),[30] erythratine (**16**),[31] erythrartine *N*-oxide (**17**),[31] erysotrine (**18**),[13] 8-oxoerythrinine (**19**),[32] 8-oxoerythraline (**20**),[13] erythraline (**21**),[13] erythraline *N*-oxide (**22**),[33] erythrinine (**23**),[20] erysovine (**24**),[21] erysodine (**25**)[34] on the basis of physical and spectrospopic comparison with published values.

Conclusions

To summary, twenty five erythrinan alkaloids were isolated from the flowers of *E. arborescens* Roxb. and among them eight novel ones, erytharborines A–H (**1–8**) have been elucidated. Alkaloids **1** and **2** were the first found erythrinan alkaloids with 2*H*-imidazole ring. In addition, **3** was an alkaloid containing an oxime group. Other alkaloids (**9–25**) were first obtained from *E. Arborescens*. The discovery of compounds **1–8** is a further addition to the diverse of alkaloids belonging to the *Erythrina* genus.

Experimental section

General experimental procedures

Optical rotations were measured with a Jasco p-1020 digital polarimeter. UV spectra were recorded on a Shimadzu 2401PC spectrophotometer. IR spectra were obtained on a Bruker Tensor 27 infrared spectrophotometer with KBr pellets. ^{1}H, ^{13}C and 2D NMR spectra were obtained on Bruker AV-600, AVANCE III-500, and AVANCE III-400 MHz spectrometers with $SiMe_4$ as an internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. ESI and HRESIMS data were recorded on a Bruker HCT/Esquire and a Shimadzu UPLC-IT-TOF spectrometer, respectively. Column chromatography (CC) was performed on either silica gel (200–300 mesh, Qingdao Marine Chemical Co., Ltd., Qingdao, China) or RP-18 silica gel (20–45 μm, YMC Chemical Ltd., Japan). Fractions were monitored by TLC on silica gel plates (GF254, Qingdao Marine Chemical Co., Ltd., Qingdao, China), and spots were visualized with Dragendorff's reagent spray. MPLC was performed using a Buchi pump system coupled with RP-18 silica gel-packed glass columns (15 × 230 and 26 × 460 mm, respectively). HPLC was performed using Waters 1525EF pumps coupled with analytical semi-preparative or preparative Sunfire C_{18} columns (4.6 × 150 and 19 × 250 mm, respectively). The HPLC system employed a Waters 2998 photodiode array detector and a Waters fraction collector III.

Plant material

Flowers of *Erythrina arborescens* Roxb. Hort. Beng were collected in September 2014 in Yunnan Province, P. R. China, and identified by Dr Chun-Xia Zeng. A voucher specimen (no. Cai20140907) was deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The dried flowers of *E. Arborescens* (6.5 kg) were powdered and extracted three times with MeOH at room temperature. After removing the solvent, the residue was dissolved in 2% HCl soln and filtered. The acidic soln was washed with EtOAc three times. The aqueous layer was then adjusted to pH 8–9 with $NH_3 \cdot H_2O$ and extracted with EtOAc to obtain crude alkaloid extract (62.5 g). The extract was subjected to column chromatography (CC) over silica gel and eluted with gradient $CHCl_3$/MeOH (1 : 0–5 : 1) to afford seven fractions (I–VII).

Fraction II (10.4 g) was further chromatographed on a C_{18} MPLC column eluted with a gradient of MeOH–H_2O (40 : 60–100 : 0, v/v) to give the five subfractions II-1–II-5. Subfraction II-2 (2.5 g) was subjected to C_{18} MPLC column once again using MeOH–H_2O (40 : 60–70 : 30, v/v) as eluent to give the four subfractions (II-2-1–II-2-4). Fraction II-2-1 was further purified by a preparative column with a gradient flow from 40% to 55% aqueous methanol to give **19** (7 mg), **8** (50 mg), **5** (5 mg). Fraction II-2-2 was separated on a preparative C_{18} HPCL column with a gradient of MeOH–H_2O (45 : 55–55 : 45, v/v) to afford **13** (4 mg) and **14** (7 mg). Fraction II-2-4 was purified by a preparative C_{18} HPCL column with a gradient of MeOH–H_2O (50 : 50–65 : 35, v/v) to obtain **11** (20 mg) and **15** (10 mg). II-4 (1.7 g) was separated using C_{18} MPLC column with a gradient of MeOH–H_2O (30 : 70–60 : 40, v/v) to afford five subfractions (II-4-1–II-4-5). Alkaloid **21** (500 mg) was crystallized from II-4-2. Fraction II-4-3 was purified by a preparative C_{18} HPCL column with a gradient of MeOH–H_2O (35 : 65–45 : 55, v/v) to obtain **22** (5 mg). II-4-5 was purified by a preparative C_{18} HPCL column with a gradient of MeOH–H_2O (50 : 50–60 : 40, v/v) to obtain **20** (5 mg). Compounds **1** (2 mg), **2** (2 mg), **3** (1.6 mg), **4** (1 mg), **9** (3 mg), **10** (2 mg) and **18** (7 mg) were obtained from fraction II-4-4 using C_{18} MPLC column with a gradient of MeOH–H_2O (40 : 60–70 : 30, v/v), then followed by preparative HPLC with a gradient of MeOH–H_2O (40 : 60–60 : 40, v/v).

Fraction III (0.9 g) was fractionated by C_{18} MPCL column with a gradient of MeOH–H_2O (30 : 70–80 : 20, v/v) to give four subfractions (III-1–III-4). III-1 was subjected to a preparative C_{18} HPCL column with a gradient of MeCN–H_2O (30 : 70–40 : 60, v/v) to afford **24** (20 mg). III-3 was further purified by a preparative C_{18} HPCL column with a gradient of MeCN–H_2O (30 : 70–45 : 55, v/v) to afford **25** (18 mg).

Alkaloid **23** (1.5 g) was crystalized from fraction IV. The mother liquid of this fraction (3.0 g) was subjected to C_{18} MPCL column with a gradient of MeOH–H_2O (20 : 80–70 : 30, v/v) to give four subfractions (IV-1–IV-4). IV-2 was separated on a preparative C_{18} HPCL column with a gradient of MeCN–H_2O (20 : 80–35 : 65, v/v) to afford **16** (12 mg), **17** (7 mg).

Fraction V (1.6 g) was chromatographed on a C_{18} MPLC column eluted with a gradient of MeOH–H_2O (20 : 80–60 : 40, v/v) to give five subfractions V-1–V-5. V-1 (910 mg) was subjected a C_{18} MPLC column once again with a gradient of MeOH–H_2O (10 : 90–40 : 60, v/v) to give eight subfractions V-1-1–V-1-8.

Compound **6** (2 mg) and **7** (2 mg) was obtained from V-1-4 using a preparative C_{18} HPCL column with a gradient of MeOH–H_2O (30 : 70–45 : 55, v/v). Compound **12** (2 mg) was obtained from V-1-6 using a preparative C_{18} HPCL column with a gradient of MeOH–H_2O (40 : 60–50 : 50, v/v).

Erytharborine A (1)

Pale yellow amorphous powder; $[\alpha]_D^{25}$ + 119.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.03), 227 (3, 79), 289 (3.55), 322 (3.48) nm; IR (KBr) ν_{max} 2927, 1710, 1629, 1479, 1383, 1252 cm^{-1}; for ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (acetone-d_6), see Tables 1 and 2; positive HRESIMS *m*/*z* 380.1961 $[M + H]^+$ (calcd. For $C_{22}H_{26}N_3O_3$, 380.1969).

Erytharborine B (2)

Pale yellow amorphous powder; $[\alpha]_D^{25}$ + 377.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 201 (3.99), 230 (3.80), 288 (3.59), 327 (3.50), nm; IR (KBr) ν_{max} 3429, 2930, 1722, 1633, 1594, 1508, 1479, 1392, 1252 cm^{-1}; for ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (acetone-d_6), see Tables 1 and 2; positive HRESIMS *m*/*z* 364.1658 $[M + H]^+$ (calcd. For $C_{21}H_{22}N_3O_3$, 364.1656).

Erytharborine C (3)

White powder; $[\alpha]_D^{23}$ + 112.2 (*c* = 0.25, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 204 (4.22) and 289 (3.46) nm; IR (KBr) ν_{max} 3414, 2931, 1611, 1513, 1458, and 1256 cm^{-1}; for ^1H (600 Hz) and ^{13}C (150 Hz) NMR data (DMSO-d_6), see Tables 1 and 2; positive ESIMS *m*/*z* 427 $[M + H]^+$, HRESIMS *m*/*z* 427.1197 $[M + H]^+$ (calcd. For $C_{20}H_{25}N_2O_4Cl_2$, 427.1191).

Erytharborine D (4)

White powder; $[\alpha]_D^{22}$ + 63.6 (*c* = 0.14, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 206 (3.68), 232 (3.08) and 283 (2.69) nm; ^1H (600 Hz) and ^{13}C (150 Hz) NMR data (acetone-d_6), see Tables 1 and 2; positive ESIMS *m*/*z* 398 $[M + H]^+$, HRESIMS *m*/*z* 398.1281 $[M + H]^+$ (calcd. For $C_{20}H_{26}NO_3Cl_2$, 398.1284).

Erytharborine E (5)

Colorless oil; $[\alpha]_D^{22}$ + 179.8 (*c* = 0.19, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 203 (3.93), 223 (3.42) and 283 (2.95) nm; ^1H (600 Hz) and ^{13}C (150 Hz) NMR data (acetone-d_6), Tables 1 and 2; positive ESIMS *m*/*z* 330 $[M + H]^+$, HRESIMS *m*/*z* 330.1699 $[M + H]^+$ (calcd. For $C_{19}H_{24}NO_7$, 330.1700).

Erytharborine F (6)

White powder; $[\alpha]_D^{22}$ + 111.5 (*c* = 0.18, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 203 (3.63), 248 (3.33), 289 (3.13) and 352 (2.94) nm; ^1H (600 Hz) and ^{13}C (150 Hz) NMR data (acetone-d_6), Tables 1 and 2; positive ESIMS *m*/*z* 398 $[M + Na]^+$, HRESIMS *m*/*z*. 398.1212 $[M + Na]^+$ (calcd. For $C_{19}H_{21}NO_7Na$, 398.1210).

Erytharborine G (7)

White powder; $[\alpha]_D^{22}$ + 311.1 (*c* = 0.05, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 204 (4.33), 225 (3.80), and 283 (3.29) nm; ^1H (600 Hz) and ^{13}C (150 Hz) NMR data (acetone-d_6), Tables 1 and 2; positive ESIMS *m*/*z* 384 $[M + Na]^+$, HRESIMS *m*/*z*. 384.1417 $[M + Na]^+$ (calcd. For $C_{19}H_{23}NO_6Na$, 384.1418).

Erytharborine H (8)

Colorless oil; $[\alpha]_D^{22}$ + 161.1 (*c* = 0.25, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 204 (3.91), 241 (3.47) and 283 (2.88) nm; ^1H (400 Hz) and ^{13}C (125 Hz) NMR data (acetone-d_6), Tables 1 and 2; positive ESIMS *m*/*z* 366 $[M + Na]^+$, HRESIMS *m*/*z* 366.1310 $[M + Na]^+$ (calcd. For $C_{19}H_{21}NO_5Na$, 366.1312).

Conflicts of interest

There are no conflicts to declare.

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