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Erythrina alkaloids from leaves of *Erythrina arborescens*

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ABSTRACT

Continued interest in *Erythrina* alkaloids resulted in the isolation of 38 alkaloids including 7 undescribed ones from the leaves of *Erythrina arborescens* Roxburgh. Among the new compounds, erythrivarines H-I were two dimeric alkaloids, while others were *Erythrina* alkaloid glucosides. Dimeric *Erythrina* alkaloids and monomers, turcomanidine and isoboldine, showed medium xanthine oxidase inhibition.

1. Introduction

The genus *Erythrina* L. (Fabaceae) comprises more than 100 species and is mainly distributed in tropical and subtropical areas. Among them, there are four local and two introduced species occurring in China [1]. To date, more than 100 alkaloids are reported from this genus but reports of glycosylated alkaloid derivates are scant. The reported bioactivities and relatively conservative structures of *Erythrina* alkaloids had not promoted themselves to be a phytochemical hot field. Nevertheless, they have gained recently attention of chemists due to their distinct spirocyclic skeleton [2]. Polymerization, a special form of natural product genesis, not only diversifies molecules but also affects the physicochemical and pharmacological properties of compounds. In addition, glycosidation is another important way to form diverse natural products. As far as *Erythrina* alkaloids, there are rarely reported glycosylated alkaloids. The known individual *Erythrina* alkaloid mainly could affect psychosis system, such as anxiolytic-like activity [3]. In addition, like colchicine derived from phenylalanine, does *Erythrina* alkaloid possess antigout activity? Our previous studies on the alkaloids from Yunnan local plant resources disclosed the first dimeric and trimeric *Erythrina* alkaloids from the follower of *Erythrina variegata* Linn. [3]· [4] Hence, this finding attracted us to study the closely related species *Erythrina arborescens* Roxburgh. As a result, 25 alkaloids including eight undescribed ones were obtained from its flowers [5]. As part of our contained research on E. arborescens, 7 undescribed alkaloids, named as erythrivarines H-I (**1**–**2**), erythraline-11-*O*-*β*-D-glucose (**3**), erythrartine-11-*O*-β-D-glucose (**4**), erythraline *N*-oxide-11-*O*-*β*-D-glucose (**5**), 10-oxo-erythraline-11-*O*-*β*-D-glucose (**6**), 10-oxo-erythrartine-11-*O*-*β*-D-glucose (**7**), together with 31 known ones were described from its leaves in the present work.

2. Results and discussion

Compounds **1**–**7** (Fig. 1) might be alkaloids as they showed positive reaction with Dragendorff's reagent on TLC plates. The UV absorption of **1** at 202, 232, and 280 nm indicated a tetrahydroisoquinoline chromophore [6]. Furthermore, its IR absorption bands at 1730, 1489, 1452 cm^{-1} resulted from the carbonyls and aromatic rings, which was consistent with the characteristics of

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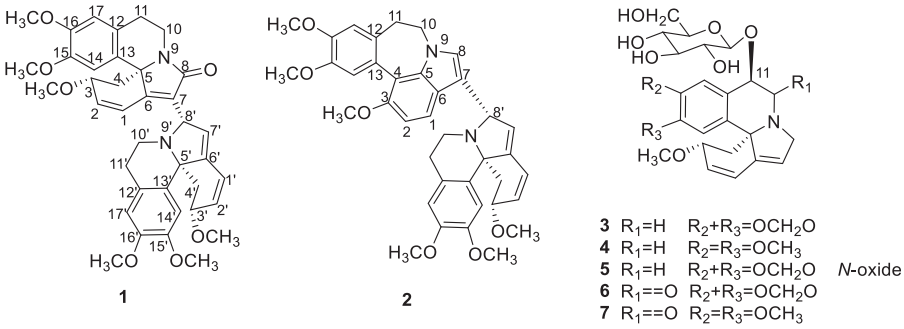

Fig. 1. New alkaloids from leaves of *Erythrina arborescens*.

Erythrina alkaloid. The HRESIMS (m/z = 661.2881 $[M + Na]^{+\cdot}$) and ^{13}C NMR spectroscopic data (Table 1) of **1** established the molecular formula $C_{38}H_{42}N_2O_7$. In the ^1H NMR spectrum of **1** (Table 1), six singlets (δ_H 6.94, 6.90, 6.88, 6.70, 5.63, 4.76), four doublets (δ_H 7.65, dd, J = 10.8, 2.4 Hz), 6.34 (d, J = 10.8 Hz), 6.59 (dd, J = 10.2, 2.4 Hz), 6.11 (d, J = 10.2 Hz), and six methoxyl groups (δ_H 3.83, 3.78, 3.71, 3.69, 3.31 × 2) indicated that **1** might be a dimeric *Erythrina* alkaloid. The ^{13}C NMR spectrum (Table 1) of **1** displayed 14 quaternary carbons (δ_C 171.4, 153.0, 149.8, 149.0, 148.3, 148.1, 142.6, 132.1, 130.7, 129.9, 127.8, 127.5, 68.1, 65.9), 12 methines (δ_C 136.0, 133.3, 126.3, 125.7, 124.4, 113.6, 113.2, 110.8, 109.8, 77.1, 75.7, 63.4), six methylenes (δ_C 42.5, 42.0, 41.8, 38.2, 27.2, 24.1) and six methoxyl groups (δ_C 56.4, 56.4, 56.3, 56.1, 56.0, 55.9). The above mentioned data suggested that **1** was similar to the previously reported dimeric erythrivarine A [3] except that two methylenedioxys were in erythrivarine A, instead, four additional methoxyls were present in **1**. Those differences suggested **1** possessed two *ortho*-methoxy groups which with further confirmed by the two pair of aromatic singlets in its ^1H NMR spectrum. The HMBC correlations from two pairs of methylene protons (δ_H 3.21 and 2.98, and δ_H 2.86 and 2.52) to C-17/17′ (δ_C 113.6 d and 113.2 d) assigned the methylenes to CH_2-11/11′ in **1**, respectively, rather than methines in the erythrivarine A. Further comparison with the ^{13}C NMR spectra of erythrivarine A, the newly carbonyl signal (δ_C 171.4 s) in **1** was assigned to C-8 based on the HMBC correlations from H-10 to C-5 and C-8. Another methine proton 4.76 (s) showed the HMBC correlations to C-8 (δ_C 171.4 s), C-6 (δ_C 153.0 s), C-6' (δ_C 142.6 s), and C-10' (δ_C 41.8 t), assigning itself to H-8 and connectivity via C-7/8′ between both units (Fig. 2).

Two typical C-3(3′) methoxyl groups were supported by the HMBC correlations of δ_H 3.85 (H-3)/δ_C 65.9 (C-5), 124.4 (C-1), 56.4 (OMe), and of δ_H 4.11 (H-3′)/δ_C 68.1 (C-5′), 125.7 (C-1′), and 56.4. The methoxy group at C-3 (3′) was determined as α-oriented through the ROESY correlations of H-3 (3′)/H-14 (14′), and the large coupling constants of H-4/4'. Though there were no NOEs with H-8′, H-8′ was deduced to be α-configuration which was its preferred conformation in combination with the X-ray diffraction of unit, erythrinine [3]. The optical rotation of alkaloid **1** ($[a]_D^{25}$ −160.9), was closed to that of erythrivarine A ($[a]_D^{25}$ −165)[3], indicating the identical stereo-configuration. In addition, the configuration of C-5 was

Table 1
^1H (600 MHz) and ^{13}C (150 MHz) NMR data of alkaloids **1** and **2** in acetone-d_6 (δ in ppm, J in Hz).

Entry	δ_H (1)	δ_C (1)	δ_H (2)	δ_C (δ_C (2))
1	7.65, dd (10.8, 2.4)	124.4 d	7.63, d (9.0)	120.6 d
2	6.34, d (10.8)	136.0 d	6.94, d (9.0)	108.4 d
3	3.85, overlap	75.7 d		153.6 s
4	2.92, dd (10.8, 4.8)	42.0 t		113.6 s
	1.57, t (10.8)			
5		65.9 s		135.6 s
6		153.0 s		124.8 s
7		129.9 s		114.6 s
8		171.4 s	7.08, s	129.7 d
10	3.87, m	38.2 t	4.34, brs (2H)	53.5 t
	3.60, m			
11	3.21, overlap	27.2 t	3.07, t (4.8, 2H)	36.0 t
	2.98, overlap			
12		127.8 s		126.8 s
13		130.7 s		134.5 s
14	6.94, s	109.8 d	7.55, s	117.3 d
15		149.0 s		147.8 s
16		149.8 s		148.9 s
17	6.90, s	113.6 d	6.86, s	113.1 d
3-OCH_3	3.31, s (3H)	56.4 q	3.85, s (3H)	57.7 q
15-OCH_3	3.83, s (3H)	55.9 q	3.86, s (3H)	56.0 q
16-OCH_3	3.78, s (3H)	56.0 q	3.84, s (3H)	55.9 q
1′	6.59, dd (10.2, 2.4)	125.7 d	6.64, d (10.2)	126.0 d
2′	6.11, d (10.2)	133.3 d	6.13, d (10.2)	133.0 d
3′	4.11, m	77.1 d	4.17, m	77.1 d
4′	2.63, dd (10.8, 6.0)	42.5 t	2.49, dd (12.0, 6.0)	4 42.7 t
	1.92, t (10.8)		1.95, t (12.0)	
5′		68.1 s		67.7 s
6′		142.6 s		142.4 s
7′	5.63, s	126.3 d	5.69, s	128.9 d
8′	4.76, s	63.4 d	4.97, s	63.4 d
10′	3.30, overlap	41.8 t	3.25, m	41.1 t
	2.75, m		2.92, overlap	
11′	2.86, overlap	24.1 t	3.05, m	23.9 t
	2.52, m		2.50, dt (16.2, 3.7)	
12′		127.5 s		127.6 s
13′		132.1 s		132.8 s
14′	6.88, s	110.8 d	6.96, s	110.9 d
15′		148.1 s		148.2 s
16′		148.3 s		149.2 s
17′	6.70, s	113.2 d	6.75, s	113.2 d
3′-OCH_3	3.31, s (3H)	56.4 q	3.35, s (3H)	56.3 q
15′-OCH_3	3.71, s (3H)	56.3 q	3.72, s (3H)	56.1 q
16′-OCH_3	3.69, s (3H)	56.1 q	3.83, s (3H)	56.0 q

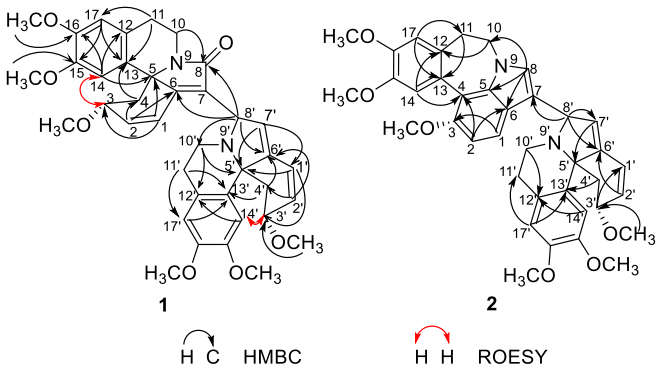

Fig. 2. The key HMBC correlations of alkaloids **1** and **2**.

S in all reported *Erythrina* alkaloids. Besides that, the absolute configuration of **1** was determined to be 3(3′)*R*,5*R*,5′*S*,8′*S*. All signals of ^{1}H and ^{13}C NMR were assigned by HSQC, HMBC spectra.

Alkaloid **2** was obtained as a yellow powder, which was consistent with the UV absorptions at 312 and 438 nm in its UV spectrum. The similar UV spectra of **2** to the previous reported erythrivarine B[3] indicated a similar conjugated system for both compounds. The HRESIMS (*m/z* 621.2955) of **2** suggested the molecular formula as $C_{38}H_{40}N_2O_6$ $[M+H]^+$, an additional degree of unsaturation than **1**. Furthermore, compared to the ^{13}C NMR data of erythrivarine B, two methylenedioxy groups in erythrivarine B[3] were substituted by four additional methoxyl groups in **2**, which were confirmed by the HMBC crosspeaks. Additionally, the CH-11/11′ (ab. δ_C 70) in erythrivarine B were substituted by two methylene groups in **2**, suggesting the absence of hydroxyl groups at C-11/11′ in **2**. This presumption was supported by the HMBC correlations from δ_H 6.86 (H-17) to δ_C 36.0 (C-11) and from δ_H 6.75 (H-17′) to δ_C 23.9 (C-11′). Its stereo-configuration of **2** was identical to that of erythrivarine B based on their biosynthesis and close optical rotations. The co-occurrence of **1** and **2** further the supported presumption of structure and biosynthetic relationship from erythrivarine A to erythrivarine B as stated in the previous study [3]. Thus **1** and **2** were subsequently named as erythrivarines H and I because of the previously reports of the dimeric and trimeric erythrivarines A-G [3,4].

Compounds **3**–**7** were obtained as white powders. Their UV and IR spectra showed characteristic of *Erythrina* alkaloids. Their ^{1}H and ^{13}C NMR displayed a β-glucose unit on the basis of each proton signal at δ_H 4.5–4.9 (d, J = ab. 8.0 Hz) and an anomeric carbon [ab. δ_C 104 (d)], a methylene at ab. δ_C 62, and the four methine signals between δ_C 69 and δ_C 80. The identification of the sugar residues were continued by hydrolysis with 10% HCl to afford D-glucose which were confirmed by comparison with determination of their optical rotation values ($[\alpha]_D^{21}$ = +46.5°, +48.0°, +47.4°, +54.9°, +46.2°) [7].

The HR-ESI-MS of **3** at *m/z* = 498.1733 $[M+H]^{+\cdot}$) in combination with its ^{1}H and ^{13}C NMR spectra gave a molecular formula $C_{24}H_{29}NO_9$. Besides a sugar unit, its ^{1}H NMR spectrum (Table 2) indicated 3 singlets at δ_H 7.21, 6.61 and 5.75; two sp [2] doublets δ_H 6.55 (dd, J = 10.2, 1.2 Hz), 5.99 (d, J = 10.2 Hz), one methoxy δ_H 3.22 (3H, s), one methylenedioxy at δ_H 5.97 and 5.95 (each 1H, s), similar to those of erythrinine [see supporting information]. The ^{13}C NMR spectrum of **3** (Table 3) displayed six quaternary carbons, seven methines, three methylenes, very same to erythrinine. In the HMBC spectrum, correlations from H-11 (δ_H 4.59, overlap) to δ_C 104.3 (d, C-1′) and 108.1 (d, C-17) confirmed glucosyl moiety connected to C-11. The ROESY correlations of δ_H 3.78 (H-3)/δ_H 6.61 (H-14) and of δ_H 4.59 (H-11)/δ_H 2.35 (H-4) placed 3-OMe and H-11 at α-orientation.

Molecular formula of alkaloid **4** was determined to $C_{25}H_{33}NO_9$ through its HR-ESI-MS (*m/z* 492.2226 $[M+H]^{+\cdot}$) with 10° of unsaturation. The ^{1}H and ^{13}C NMR (Tables 2 and 3) were very close to those of **3**, except the two additional methoxyls δ_H 3.80 (3H, s) and 3.70 (3H, s). The HMBC correlations of δ_H 3.80 (3H, s)/δ_C 149.6 (s, C-15) and of δ_H 3.70 (3H, s)/δ_C 149.0 (s, C-16) supported additional methoxyls at C-15/16 in **4** rather than the methylenedioxy in **3**.

Molecular formula of alkaloid **5** was determined to $C_{24}H_{29}NO_{10}$ by HR-ESI-MS (*m/z* = 514.1685 $[M+Na]^+$), showing one additional oxygen atom than **3**. The ^{1}H and ^{13}C NMR (Tables 2 and 3) shift values of **5** were very similar to those of **3**, with exception for three downfield signals (δ_C 72.9, 63.3, 81.0) in the ^{13}C NMR spectrum. The differences between **3** and **5** suggested **5** was *N*-oxide derivative of **3**. The molecular formula $C_{24}H_{27}NO_{10}$ of **6** was elucidated on the basis of the HRESIMS *m/z* 512.1531 ($[M + Na]^+$). Compared to that of **3**, the ^{13}C NMR spectrum of **6** indicated an additional carbonyl signal (δ_C 172.7), instead of one of the methylenes in **6**. The HMBC correlations from δ_H 5.75 (H-11) to δ_C 133.0 (C-13), 103.2 (C-1′) and 172.7 placed the carbonyl signal at C-10. Alkaloid **7** possessed the molecular formula of $C_{25}H_{31}NO_{10}$ based on the HRESIMS *m/z* 528.1844 ($M + Na]^{+\cdot}$). Comparison of the NMR spectra obtained

Table 2
^{1}H (600 MHz) NMR data of alkaloids **3**–**7** (δ in ppm, J in Hz).

Entry	δ_H (3)	δ_H (4)	δ_H (5)	δ_H (6)	δ_H (7)
1	6.55, dd (10.2, 1.2)	6.58, dd (10.2, 2.4)	6.64, dd, 10.2, 1.8	6.74, (d 10.2)	6.76, dd (10.2, 1.8)
2	5.99, d (10.2)	6.03, d (10.2)	6.12, d, 10.2	6.04, d (10.2)	6.06, d (10.2)
3	3.78, m	4.04, m	4.07, m	3.70, m	3.72, m
4	2.35, dd (12.0, 5.4)	2.42, dd (11.4, 5.4)	2.92, overlap	2.67, dd (11.4, 5.4)	2.69, dd (11.4, 4.8)
	1.53, t (12.0)	1.69, t (11.4)	1.92, overlap	1.87, t (11.4)	1.88, t (11.4)
7	5.75, s	5.70, s	5.82, s	5.92, s	5.90, s
8	3.67, d (15.6)	3.87, d (15.6)	4.87, overlap	4.33, d (10.2, 2H)	4.33, d (10.2, 2H)
	3.58, d (15.6)	3.68, overlap	3.95, dd (15.6, 3.0)		
10	3.44, overlap	3.92, dd (12.0, 3.0)	4.20, dd (13.0, 2.0)		
	2.95, dd (13.2, 4.8)	3.72, overlap	3.75, overlap		
11	4.59, overlap	4.67, overlap	4.90, overlap	5.75, s	5.74, s
14	6.61, s	6.85, s	6.54, s	6.91, s	7.03, s
17	7.21, s	7.20, s	7.03, s	7.35, s	7.56, s
3-OCH_3	3.22, s (3H)	3.29, s (3H)	3.29, s (3H)	3.18, s (3H)	3.24, s (3H)
15-OCH_3		3.70, s (3H)			3.74, s (3H)
16-OCH_3		3.80, s (3H)			3.84, s (3H)
OCH_2O	5.97, s		6.06, s	6.02, s	
	5.95, s		6.02, s	6.00, s	
1′	4.47, d (8.4)	4.69, overlap	4.57, d (7.8)	4.85, d (8.4)	4.85, d (7.2)
2′	3.18, overlap	3.40, overlap	3.32, overlap	3.46, m	3.48, m
3′	2.99, m	3.27, overlap	2.95, overlap	3.48, m	3.50, m
4′	3.05, m	3.27, overlap	3.04, m	3.44, m	3.51, m
5′	3.16, overlap	3.28, overlap	3.23, overlap	3.43, m	3.49, m
6′	3.71, m	3.48, m	3.74, overlap	3.85, d (10.8)	3.85, overlap
	3.47, overlap	3.39, overlap	3.45, overlap	3.70, m	3.70, overlap
2′-OH	5.25, d (5.4)	3.54, d (4.8)	3.73, overlap		
3′-OH	5.02, d (4.8)	3.52, d (4.2)	3.31, overlap		
4′-OH	4.56, overlap	3.30, d (3.0)	3.21, overlap		
6′-OH	4.98, d (5.4)	3.33, d (3.6)	3.30, overlap		

Alkaloids **3** and **5** were recorded in DMSO-d_6, **4, 6** and **7** in acetone-d_6.

Table 3
^{13}C (150 MHz) NMR data of alkaloids **3**–**7** (δ in ppm, J in Hz).

Entry	δ_C (3)	δ_C (4)	δ_C (5)	δ_C (6)	δ_C (7)
1	124.9 d	126.0 d	125.5 d	125.0 d	125.0 d
2	131.3 d	132.4 d	132.5 d	132.3 d	132.5 d
3	75.6 d	77.0 d	75.0 d	76.9 d	77.1 d
4	41.3 t	41.8 t	30.0 t	41.1 t	41.1 t
5	66.4 s	66.9 s	81.0 s	72.0 s	72.0 s
6	141.6 s	143.2 s	137.9 s	139.6 s	139.7 s
7	123.7 d	124.3 d	119.9 d	121.2 d	121.0 d
8	59.1 t	59.1 t	72.9 t	55.0 t	54.9 t
10	50.4 t	50.2 t	63.3 t	172.7 s	172.6 s
11	72.7 d	74.0 d	73.8 d	71.0 d	71.8 d
12	129.0 s	127.7 s	124.6 s	129.6 s	127.6 s
13	131.5 s	131.5 s	129.4 s	133.0 s	131.3 s
14	104.6 d	109.7 d	104.3 d	104.4 d	108.3 d
15	146.5 s	149.6 s	147.2 s	147.7 s	148.9 s
16	145.9 s	149.0 s	148.1 s	148.2 s	149.9 s
17	108.1 d	113.2 d	109.0 d	107.6 d	110.7 d
3-OCH_3	55.7 q	56.2 q	55.9 q	56.3 q	56.3 q
15-OCH_3		56.0 q			56.3 q
16-OCH_3		56.0 q			56.2 q
OCH_2O	100.8 t		101.7 t	102.6 t	
1′	104.3 d	105.6 d	104.3 d	103.2 d	103.6 d
2′	77.0 d	78.0 d	76.9 d	78.6 d	78.4 d
3′	73.9 d	75.2 d	73.8 d	77.3 d	77.5 d
4′	70.2 d	71.6 d	70.1 d	72.9 d	73.3 d
5′	76.9 d	77.6 d	77.3 d	70.7 d	71.0 d
6′	61.3 t	63.0 t	61.3 t	62.6 t	62.5 t

Alkaloids **3** and **5** were recorded in DMSO-d_6, **4, 6** and **7** in acetone-d_6.

from **7** and **6** showed that the differences between them were caused by a methoxyl group in **7**, instead the methylenedioxy in **6**.

Due to the structure similarities, **3**–**7** were named as erythraline-11-*O*-β-D-glucose (**3**), erythrartine-11-*O*-β-D-glucose (**4**), erythrartine *N*-oxide-11-*O*-β-D-glucose (**5**), 10-oxo-erythraline-11-*O*-β-D-glucose (**6**) and 10-oxo-erythrartine-11-*O*-β-D-glucose (**7**), respectively.

The other known compounds were determined as 10-oxo-erythraline (**8**), erymelanthine (**9**), erytharbine (**10**), 8-oxo-erythraline (**11**), 10, 11-dioxoerysotrine (**12**), 8-oxo-erythrinine (**13**), 10-hydroxy-11-oxoerysotrine (**14**), erysotramidine (**15**), crystamidine (**16**), 10,11-dioxoerythraline (**17**), norreticuline (**18**), erybidine (**19**), erysothrine (**20**), turcomanidine (**21**), erythraline (**22**), cristanine A (**23**), erythrinine (**24**), 11-hydroxyerysotrine (**25**), isoboldine (**26**), erythrivarine B (**27**), erythriarborine B (**28**), β-erythroidine (**29**), 11β-hydroxyerythratidine (**30**), erythrinine *N*-oxide (**31**), 8-oxo-11β-methoxyerythraline (**32**), erythrartine *N*-oxide (**33**), 11β-hydroxyerysotramidine (**34**), 8-oxo-11β-methoxyerysodine (**35**), 11β-methoxyerysotrine (**36**), erytharborine A (**37**), erytharborine B (**38**) based on their NMR spectra and MS data.

All alkaloids were evaluated for their cytotoxicity against breast cancer (MCF-7), colon cancer (SW480), and HeLa cells using the MTT method. However, they did not show activities ($IC_{50} > 20$ μg/mL). Alkaloids **1**–**38** were screened for the xanthine oxidase (XO) inhibitory activities, with allopurinol as the positive control. The obtained IC_{50} values inhibition activity of **1**–**2**, **21, 26, 37**–**38** were given in Table 4.

Table 4
Xanthine oxidase inhibition activities of **1**–**2**, **21, 26, 37** and **38.**

Compound	IC_{50} (μg/mL)
1	5.3
2	4.6
21	2.7
26	2.4
37	4.1
38	4.2
Allopurinol	0.60

3. Experimental section

3.1. General information

Optical rotations were measured with either a Horiba SEPA-300 polarimeter (Horiba Scientific, Kyoto, Japan) or JASCO DIP-370 digital polarimeter (Jasco International Co., Tokyo, Japan). UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer (Shimadzu Corp., Kyoto, Japan). Scanning IR spectroscopy was performed on a Tenor 27 spectrophotometer using KBr pellets. MS data were recorded on an Agilent G6230 TOF MS (Applied Biosystems, Ltd., Warrington, UK). 1D- and 2D- NMR spectra were obtained on Bruker Avance III-600, DRX-500, and AM-400 spectrometers (Bruker BioSpin GmBH, Rheinstetten, Germany) using TMS as an internal standard. Column chromatography (CC) was performed on silica gel (200–300 mesh, Qing-dao Haiyang Chemical Co., Ltd, Qingdao, China) and C_{18}-silica gel (20–45 μm, Fuji Silysia Chemical Ltd.). Fractions were analyzed by TLC on silica gel plates (GF254, Qingdao Haiyang Chemical Co., Ltd.) and spots visualized with Dragendorff's reagent. Medium pressure liquid chromatography (MPLC) was employed using a Buchi pump system coupled with C_{18}-silica gel-packed glass column (15 × 230 and 26 × 460 mm). High performance liquid chromatography (HPLC) was performed using a Waters 600 pump (Waters Corp., Milford, MA, USA) coupled with Sunfire analytical, or preparative Sunfire, Xbridge, and Cosmosil C18 columns (150 × 4.6, and 250 × 19(20) mm, respectively). The HPLC analyses were performed on a e 1525 EF Waters instrument coupled with 2998 photodiode array detector and a Waters fraction collector II (Waters Corp.).

3.2. Plant material

Leaves of *Erythrina arborescens* Roxburgh 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.

3.3. Extraction and isolation

Dried leaves of *E. arborescens* (19 kg) were powdered and extracted three times with MeOH at room temperature. After removing the solvent, the residue was dissolved in 0.5% HCl soln. and filtered. The acidic soln. was washed with EtOAc three times. The aqueous layer was then adjusted to pH 7–8 with $NH_3 \cdot H_2O$ and subsequently extracted with EtOAc to obtain crude alkaloid extract (85 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 nine fractions (I–IX). Base water layer was loaded on D101 resin column eluded with H_2O, then MeOH. Concentrated MeOH washer (200 g) was subjected to Silica gel column and CH_3Cl-acetone (from 1:0 to 0:1), to give six Fractions (Fr. X-XV).

Fraction I (4.5 g) was further chromatographed on a C_{18} MPLC column eluted with a gradient of MeOH-H_2O(30%–80%)to give 3 subfraction I-1~I-3. Alkaloid **12** (30 mg) was crystalized from Subfraction I-1. Mother liquid of I-1 (0.5 g) was separated by C_{18} MPLC column, eluting with MeOH-H_2O (30%–80%) to yield 3 mixtures I-1a~I-1c. I-1a was purified by C18 Xbridge HPLC with MeOH-H_2O (45%–60%) to obtain **14** (1 mg). I-1b was purified on Sunfire C18 HPLC (5 μm, 20 × 250 mm) with MeOH-H_2O (25%–40%) to give **17**

(3 mg). I-1c was purified by the same column with acetonitrile −H_2O (15%–40%) to give **15** (116 mg). I-2 (0.8 g) was further purified on the C_{18} MPLC column with a gradient flow from 20% to 80% aqueous methanol to give I-2a and I-2b. I-2a was separated on a preparative Sunfire column with a gradient of MeOH-H_2O (45%–60%) to afford **10** (46 mg) and **11** (45 mg). Same method was used to purify I-2b with acetonitrile–H_2O (30%–45%) to obtain **15** (152 mg). I-3 (0.5 g) was loaded on the C_{18} MPLC column with a gradient flow from 20% to 80% aqueous methanol to give (20%–80%) to give I-3a and I-3b. I-3a was separated by preparative Xbridge C18 column eluted with aqueous methanol (45%–60%) to yield **8** (28 mg). I-3b was separated on a preparative Sunfire C_{18} column with aqueous methanol (45%–60%) to get **16** (15 mg). Fraction II (2.2 g) was purified on a C18 silica gel CC with aqueous methanol (30%–80%, v/v) to afford three subfractions (II-1~II-3). Subfraction II-1 (0.5 g) was separated by C_{18} MPLC column with a gradient of MeOH-H_2O (15%–80%) to II-1a and II-1b. II-1a was purified by Cosmosil C18 (5 μm, 20 × 250 mm) HPLC column with a gradient of MeOH-H_2O (55%–70%) to afford **37** (3 mg). II-1b was separated using C_{18} MPLC column using acetonitrile–H_2O (10%–50%) to give **38** (2 mg). II-2 (0.2 g) was separated by Sephadex LH-20, eluting with MeOH-H_2O (10%–40%) to yield II-2a and II-2b. II-2a was purified on Cosmosil C18 column, MeOH-H_2O (50%–75%) to give **9** (1 mg). II-3 (0.8 mg) was separated by RP-18 with acetonitrile −H_2O (10%–60%), and then purified on Xbridge C18 with acetonitrile–H_2O (50%–65%) to obtain **1** (3 mg). Fraction III (5.0 g) was subject to C18 silica gel CC with MeOH-H_2O (20%–80%) to give III-1~III-2. Alkaloid **13** (93 mg) was crystalized from III-1. Fraction IV (5.0 g) was further separated by C_{18} MPLC column with a gradient of MeOH-H_2O (20%–80%) to give four subfractions IV-1~IV-4. IV-1 (0.5 g) separated by C_{18} MPLC column with a gradient of MeOH-H_2O (25%–40%) to give **18** (6 mg). Subfraction IV-2 (0.2 g) was loaded on the Sephadex LH-20 column with MeOH-H_2O (30%–50%) to give **19** (20 mg). Subfraction IV-3 (0.5 g) was separated by C_{18} MPLC column with a gradient of MeOH-H_2O (20%–80%) to give IV-3a and IV-3b. IV-3a was purified on Sunfire C18 with a gradient of MeOH-H_2O (40%–50%) to yield **4** (38 mg). Subfraction IV-3b was purified with same method to give **5** (2 mg). IV-4 was purified by same column with MeOH-H_2O (45%–55%) to give **3** (1.9 mg). Fraction V (7.1 g) was subjected to C_{18} MPLC column with a gradient of MeOH-H_2O (20%–80%) to give 3 Subfraction V-1~V-3. Subfraction V-1 (0.2 g) was subjected to C_{18} MPLC column again with MeOH-H_2O (10%–80%) and then purified by Sunfire C18 column with MeOH-H_2O (45%–60%) to get **23** (10 mg). Subfraction V-2 (2.5 g) subjected to C_{18} MPLC column again with acetonitrile–H_2O (20%–80%) give **20** (700 mg). Subfraction V-3 (0.8 g) was separated on C_{18} MPLC column again with MeOH-H_2O (30%–80%) to give V-3a and V-3b. V-3a (0.3 g) was separated on the C_{18} MPLC column with MeOH-H_2O (10%–60%) to afford **22** (300 mg). Fraction VI (3.0 g) was separated on C_{18} MPLC column with MeOH-H_2O (20%–80%) to give **21** (42 mg). Fraction VII (8.0 g) was separated on C_{18} MPLC column with MeOH-H_2O (20%–80%) to give subfraction VII-1 and VII-2. Subfraction VII-1 was purified on Xbridge C18 column with MeOH-H_2O (45%–60%) to give **25** (50 mg). Alkaloid **24** (600 mg) was crystalized from VII-2. Fraction VIII (0.4 g) was separated on Sephadex LH-20 column with MeOH-H_2O (30%–50%) to give **19** (1 mg). Fraction IX (4.8 g) was separated on C_{18} MPLC column with MeOH-H_2O (10%–80%), then purified on Xbridge C18 column with MeOH-H_2O (45%–60%) to give **26** (8 mg).

Fr. X (10.0 g) separated on C_{18} MPLC column with MeOH-H_2O (70%~85%) get **31** (17 mg), **9** (10 mg) and **2** (9 mg). Fr. XI (9 g) was separated by C_{18} MPLC column with 70%, 75% and 80% aqueous MeOH to yield **23** (10 mg). Fr. XII (5.2 g) was separated by C_{18} MPLC column with MeOH-H_2O (60–80%) then purified by Sunfire HPLC C18 Column with MeOH-H_2O (65–73%) to obtain **13** (420 mg) and **32** (35 mg). Fr. XIII (12 g) was separated by C_{18} MPLC column with MeOH-H_2O (50–80%) to give 4 subfractions XIII-1~XIII-4. XIII-1 was purified by Sunfire HPLC column with 50% MeOH-H_2O to give **13** (20 mg) and **32** (7 mg). XIII-3(74 mg) was separated by HPLC column MeOH-H_2O (57–67%) to obtain **12** (7 mg) and **20** (6 mg). XIII-4 (74 mg) was separated by Sunfire HPLC column with MeOH-H_2O (55–65%) to yield **6** (8 mg) and **25** (21 mg). Fr. XIIII (11 g) was separated by C_{18} MPLC column with MeOH-H_2O (50–80%). XIIII-1 (474 mg) was separated by HPLC column MeOH-H_2O (50–60%) to give **27** (5 mg). Alkaloid **24** (21 mg) was crystalized from XIIII-2. XIIII-2 (121 mg) was separated by HPLC column MeOH-H_2O (50–60%) to give **34** (13 mg) and **10** (16 mg). Fr. XV (9 g) was separated by C_{18} MPLC column with MeOH-H_2O (50–70%) to produce 3 subfractions XV-1–3. SubFr. XV-1 (160 mg) was purified by Sunfire HPLC (MeOH-H_2O:40–55%) to obtain **35** (19 mg) and **36** (26 mg). SubFr. XV-2 (130 mg) was separated by HPLC (MeOH-H_2O:50–60%) to give **7** (11 mg) and **17** (7 mg). SubFr. XV-3 (135 mg) was purified by Sunfire HPLC (MeOH-H_2O: 50–60%) to give **29** (9 mg) and **30** (7 mg). Fr. IXX (7 g) was subjected to silica gel CC with CH_3Cl-MeOH (9:1–4:1) to obtain two subfractions. IXX-1 (190 mg) was purified by Sunfire HPLC with MeOH-H_2O (45–60%) to yield **22** (9 mg). Finally, IXX-2 was purified on same column with MeOH-H_2O (50–55%) to give **32** (11 mg).

3.4. Spectroscopic data

Erythrivarine H (1): $C_{38}H_{42}N_2O_7$; white oil; $[\alpha]_D^{25}$-160.9 (*c* 0.10, MeOH); UV(MeOH) λ_{max} (logε) = 202 (4.03), 232 (3.45), 280 (3.09) nm; IR(KBr): ν_{max} cm^{-1}: 2928, 1730, 1615, 1489, 1452 cm^{-1}; ^1H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data, see Table 1; positive HRESIMS *m/z* = 661.2881 $[M + Na]^+$ (calc. for $C_{38}H_{42}N_2O_7Na$, 661.2884).

Erythrivarine I (**2**): yellow powder; $[\alpha]_D^{20}$ -138 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.05), 233 (3.83), 297 (3.28), 312 (3.30) and 438 (2.73) nm; IR (KBr) ν_{max} 2932, 1629, 1503, 1482, 1235, 1098, 1038 cm^{-1}; ^1H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data (acetone-d_6), see Table 1; positive HRESIMS *m/z* = 621.2955 $[M + H]^+$ (calc. for $C_{38}H_{40}NO_6$, 621.2959).

Erythraline 11-*O*-*β*-D-glucose (3): $C_{24}H_{29}NO_9$; white powder; $[\alpha]_D^{25}$+78.2 (*c* 0.10, MeOH); UV(MeOH) λ_{max} (logε) 202 (4.06), 232 (3.55), 286 (3.12) nm; IR(KBr) ν_{max} cm^{-1}: 3382, 3285, 3050, 1605, 1479, 1262 cm^{-1}; ^1H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data (DMSO-d_6), see Tables 2 and 3, respectively; positive HR-ESI-MS *m/z* = 498.1733 (calc. for $C_{24}H_{29}NO_9$ $[M+Na]^+$, 498.1735).

Erythrartine 11-*O*-*β*-D-glucose (4): $C_{25}H_{33}NO_9$; white powder; $[\alpha]_D^{25}$+89.6 (*c* 0.10, MeOH); UV(MeOH) λ_{max} (logε) = 204 (4.13), 235 (3.68), 285 (3.12) nm; IR(KBr): ν_{max} cm^{-1}: 3251, 2930, 1472, 1424 cm^{-1}; ^1H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data (acetone-d_6), see Tables 2 and 3, respectively; positive HR-ESI-MS *m/z* = 492.2226 (calc. for $C_{25}H_{33}NO_9$ $[M+H]^+$, 492.2228).

Erythrartine *N*-oxide-11-*O*-*β*-D-glucose (5): $C_{24}H_{29}NO_{10}$; colourless oil; $[\alpha]_D^{25}$+120.6 (*c* 0.10, MeOH); UV(MeOH) λ_{max} (logε) = 206 (4.17), 241 (3.53), 288 (3.09) nm; IR(KBr): ν_{max} cm^{-1}: 3312, 2952, 1492, 1263 cm^{-1}; ^1H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data (DMSO-d_6), see Tables 2 and 3, respectively; positive HR-ESI-MS *m/z* = 514.1685 (calc. for $C_{24}H_{29}NO_{10}Na$ $[M+Na]^+$, 514.1684).

10-Oxo-erythraline-11-*O*-*β*-D-glucose (**6**): white powder; $[\alpha]_D^{20}$ 167 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.05), 247 (3.89) and 289 (3.18) nm; IR (KBr) ν_{max} 1648, 1503, 1448 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectroscopic data (acetone-d_6), see Tables 2 and 3, respectively; positive HRESIMS *m/z* = 512.1531 $[M + Na]^+$ (calc. for $C_{22}H_{28}NO_5Na$, 512.1532).

10-Oxo-erythrartine 11-*O*-*β*-D-glucose (**7**): white powder;

$[\alpha]_D^{20}$ 141 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.04), 249 (3.88) and 289 (3.18) nm; IR (KBr) ν_{max} 3324, 2928, 1648, 1502, 1443 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectroscopic data (acetone-d_6), see Tables 2 and 3, respectively; positive HRESIMS m/z = 528.1844 $[M + Na]^+$ (calc. for $C_{18}H_{16}NO_3Na$, 528.1843).

Acid hydrolysis of 3–7. Compounds **3–7** (6 mg each) were refluxed with 10% HCl-MeOH (20 mL) at 80 °C for 6 h. After cooling, the reaction mixture was evaporated to dryness and partitioned with EtOAc. The sugars were identified as glucose by TLC comparison using MeCOEt-isoPrOH-Me_2CO-H_2O (20:10:7:6). Purification of the H_2O layer was performed by preparative TLC eluted four times with $CHCl_3$-MeOH-H_2O (70:30:1) to afford D-glucose (R_f 0.50) with positive values of specific rotation, respectively.

3.5. Cytotoxicity assay

Three human cancer cell lines, HeLa, SGC-7901 gastric cancer, and A-549 lung cancer, were used for cytotoxic assays. Cells were cultured in RPMI-1640 (Sigma-Aldrich, St. Louis, MO, USA) or DMEM medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone) in 5% CO_2 at 37 °C. Cytotoxicity assays were performed according to the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) method in 96-well microplates. Briefly, 100 μL of adherent cell types were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before the addition of test compounds. Suspended cell types were seeded at an initial density of 1×10^5 cells/mL just before drug addition. Each tumor cell line was exposed to a test compound at concentrations of 0.04, 0.20, 1.00, 5.0, and 25.0 μM in DMSO in triplicate for 48 h, with cisplatin (Sigma-Aldrich) as the positive control. After treatment, cell viability was assessed, cell growth graphed, and IC_{50} values calculated by Reed and Muench's method.

3.6. Xanthine oxidase inhibition activity

Alkaloids **1–38** were bio-assayed for inhibitory activity of xanthine oxidase. The uric acid production was calculated according to the increasing absorbance at 290 nm. Test solutions (final concentration of 50 μg/mL) were prepared by adding xanthine (final concentration 29.2 μg/mL). The reaction was started by adding 40 μL of xanthine oxidase (0.1 U/mL) in a phosphate buffer solution (pH = 7.50, 0.2 mM). Alkaloids were dissolved in DMSO and immediately diluted with phosphate buffer solution to 0.5 mg/mL. The mixture (total 100 μL) was incubated at 37 °C. The uric acid production was calculated from the differential absorbance with a blank solution in which the xanthine oxidase was replaced by buffer solution. A test mixture containing without any alkaloid was prepared to measure the total uric acid production. Different concentrations of alkaloids were analyzed, and then the half-maximal inhibitory concentration (IC_{50}) was calculated by linear regression analysis. Different concentrations of allopurinol were measured in triplicate.

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Appendix A. Supplementary data

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