Artificial *Erythrina* Alkaloids from Three *Erythrina* Plants, *E. variegata*, *E. crista-galli* and *E. arborescens*

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Abstract

Fourteen unprecedented artificial *Erythrina* alkaloids were isolated from the *Erythrina variegata*, *E. crista-galli* and *E. arborescens* (Fabaceae). The structures of these alkaloids were determined by spectroscopic analyses. Their possible formations were proposed. All isolated compounds showed no cytotoxicity and hypoglycemic activity at cell screening bioassay.

Keywords  Fabaceae · *Erythrina variegata* · *E. crista-galli* · *E. arborescens* · Artificial products · *Erythrina* alkaloids

1 Introduction

The *Erythrina*-type alkaloids with 6/5/6/6 spirocycle systems and stable 5S-chiral center are derived from two tyrosine units via oxidative coupling and intramolecular rearrangement. Since the first phytochemical research on *Erythrina* alkaloid in 1930s \([1]\), the total number now stands at well over 110 alkaloids reported from plants of *Erythrina* genus \([2]\). Bioassay screening of these alkaloids showed anxiolytic-like activity \([3]\), induced sleep \([4]\), anticonvulsant activity \([5]\), neuronal nicotinic acetylcholine receptor antagonism \([6]\), leishmanicidal \([7]\), and antifeedant activity \([8]\). Our previous research disclosed natural dimeric \([9, 10]\) and trimeric \([11]\) *Erythrina* alkaloids, alkaloidal glucosides \([10]\), and complex monomers \([12–14]\). However, during the extraction and separation of *Erythrina* alkaloids, some artificial products would be produced. Consideration of the NMR spectra characteristics and potential activities of *Erythrina* alkaloid, we here systematically summarized these artifacts from *E. arborescens*, *E. crista-galli* and *E. variegata* (Fig. 1). Their cytotoxicity against three cancer cells and hypoglycemic activity on 3T3-L1 myoblasts cell were screened. This paper will describe their isolation, structure determination and possible mechanism of formation.

2 Results and Discussion

Alkaloid 1 was obtained as white amorphous powder. Its IR absorption bands at 3441, 1640, 1503, 1480 cm\(^{-1}\) indicated the presence of the hydroxyls and aromatic rings. Moreover, the UV absorptions at 204, 239 and 290 nm indicated a tetrahydroisoquinoline chromophore \([15]\). These spectra were consistent with the characteristics of an *Erythrina* alkaloid, we here systematically summarized these artifacts from *E. arborescens*, *E. crista-galli* and *E. variegata* (Fig. 1). Their cytotoxicity against three cancer cells and hypoglycemic activity on 3T3-L1 myoblasts cell were screened. This paper will describe their isolation, structure determination and possible mechanism of formation.
an extra 2-oxopropyl group in 1. In the HMBC spectrum, correlations of \( \delta^1 H 4.20 \) (H-8) with \( \delta^1 C 53.9 \) (C-10), \( \delta^1 C 136.2 \) (C-7) and \( \delta^1 C 208.1 \) (C=O) indicated the 2-oxopropyl group at C-8. Further analysis of the 2D NMR revealed that the other parts of compound 1 were consistent with those of erythrivarine A.

Alkaloids 2 and 3 showed the same molecular formula C\(_{40}H_{42}N_2O_9\) as established by HRESIMS (\( m/z \) 695.2968 [M+H]\(^+\) in 2; \( m/z \) 695.2970 [M+H]\(^+\) in 3). In the \( ^1H \) and \( ^13C \) NMR spectra, the chemical shifts of 2 and 3 showed good agreement with those of 1, except those signals around the C-11′ position (C-10′/11′/12′). The C-11′ carbon of 1 was resonated at \( \delta^1 C 64.9 \), however, signals of the same carbon were observed at \( \delta^1 C 74.7 \) and \( \delta^1 C 74.5 \) in 2 and 3, respectively. In addition, an extra methoxyl group (\( \delta^1 H 3.54 \) in 2, \( \delta^1 H 3.36 \) in 3) was observed, which indicated that both 2 and 3 had a methoxyl group at the C-11′ position instead of a hydroxyl group in 1. Chemical shifts of H-10′, H-11′ and H-17′ protons (Table 1) of 2 and 3 implied the configuration of 11′-OCH\(_3\) in 2 and 3 were different. In the ROESY spectrum, the NOE correlation of H-3′/β/H-4′/β and H-11′/H-4′/α in 3 suggested its 11′-OCH\(_3\) was in β-orientation. The NOE correlation of H-11′/H-4′/β in 2 suggested its 11′-OCH\(_3\) was in α-orientation.

The HRESIMS of 4 showed the pseudomolecular ion at \( m/z \) 370.1651 [M+H]** (calc. for C\(_{21}H_{23}NO_5\), 370.1652). The \( ^13C \) NMR spectrum of 4 showed a methane at 64.7 ppm, which indicated the existence of hydroxy substituent. The HMBC correlation between \( \delta^1 H 7.05 \) (H-17) with \( \delta^1 C 64.9 \) suggested the hydroxy at C-11. Through detailed comparison of the 1D and 2D NMR spectrum, 4 was basically the same as erythranine [16] except for the 2-oxopropyl substituent (\( \delta^1 C 207.9, 47.8 \) and 30.8). The HMBC spectrum showed correlations from \( \delta^1 H 3.98 \) (H-8) to \( \delta^1 C 52.6 \) (C-10), \( \delta^1 C 127.6 \) (C-7) and \( \delta^1 C 207.9 \) (C=O) disclosed 4 to be 8-(2-oxopropyl)-erythranine.

Alkaloid 5 displayed a hydrogen adduct ion at \( m/z \) 354.1709 [M+H]** (calc. for C\(_{21}H_{23}NO_4\), 354.1707). The 1D NMR spectroscopic data of compound 5 were similar to those of 4 except for the following differentiations: in the \( ^1H \) NMR spectrum, the signal displayed at \( \delta^1 H 4.72 \) in 4 which was assigned to the active hydrogen in the hydroxy was disappeared in compound 5. Correspondingly, the methine signal at \( \delta^1 C 64.7 \) (C-11) in compound 4 was replaced with a methylene (\( \delta^1 C 26.0 \)) in 5. Thus, compound 5 was an analogue of 4 without the hydroxy moiety and determined to be 8-(2-oxopropyl)-erythraline.

The HRESIMS of 6 gave a hydrogen adduct ion at \( m/z \) 384.1806 [M+H]**, indicative of a molecular formula of C\(_{22}H_{25}NO_5\). In comparing with those of 4, the \( ^1H \) NMR spectrum of 6 gave signal of an additional methoxyl group (\( \delta^1 H 3.55, s, 3H \)), and its \( ^13C \) NMR spectrum showed an downfield chemical shift \( \delta^1 C 74.8 \). These findings suggested the C-11 of 6 was substituted by a methoxy rather than a hydroxy. Thus, the structure of 6 was determined to be 8-(2-oxopropyl)-11-methoxy-erythraline.

Fig. 1 Chemical structures of 14 artificial Erythrina alkaloids
Table 1  $^1$H and $^{13}$C NMR spectroscopic data for 1–3 in acetone-$d_6$ (δ in ppm and J in Hz)

| Entry | 1            | 2            | 3            |
|------|--------------|--------------|--------------|
|      | $^\delta_H$ | $^\delta_C$ | $^\delta_H$ | $^\delta_C$ | $^\delta_H$ | $^\delta_C$ |
| 1    | 7.16, dd (10.2, 4.2) | 124.6 d | 7.16, d (10.2) | 124.8 d | 7.06, overlap | 124.7 d |
| 2    | 5.92, d (10.2) | 131.1 d | 6.00, d (10.2) | 132.2 d | 6.00, d (10.2) | 132.2 d |
| 3    | 3.68, m | 76.9 d | 3.73, m | 76.8 d | 3.73, m | 77.0 d |
| 4    | 2.38, dd (11.4, 6.0) | 43.6 t | 2.44, dd (10.8, 6.0) | 42.9 t | 2.40, dd (10.8, 6.0) | 43.0 t |
|      | 1.68, t (11.4) | 1.69, t (10.8) | 1.70, t (10.8) |
| 5    | 69.2 s | 68.9 s | 69.2 s |
| 6    | 139.6 s | 139.7 s | 138.2 s |
| 7    | 136.2 d | 136.8 d | 135.6 d |
| 8    | 4.20, m | 69.7 d | 4.30, m | 68.3 d | 4.23, br s | 69.2 d |
| 9    | 3.68, m | 76.9 d | 3.73, m | 77.0 d |
| 10   | 2.38, dd (11.4, 6.0) | 43.6 t | 2.44, dd (10.8, 6.0) | 42.9 t | 2.40, dd (10.8, 6.0) | 43.0 t |
|      | 1.68, t (11.4) | 1.69, t (10.8) | 1.70, t (10.8) |
| 11   | 4.65, m | 64.7 d | 4.66, m | 64.7 d | 4.75, m | 64.8 d |
| 12   | 134.1 s | 133.9 s | 133.9 s |
| 13   | 132.2 s | 132.6 s | 132.6 s |
| 14   | 6.74, s | 106.1 d | 6.73, s | 105.8 s | 6.90, s | 106.1 d |
| 15   | 147.7 s | 147.3 s | 147.3 s |
| 16   | 147.2 s | 147.3 s | 147.3 s |
| 17   | 7.01, s | 108.7 d | 7.01, s | 108.6 d | 7.06, s | 107.3 d |
| OCH$_2$O | 5.96, s | 101.8 t | 5.96, s | 101.8 t | 5.96, s | 102.0 t |
| 3-OCH$_3$ | 5.94, s | 5.95, s | 5.95, s |
| 11-OH | 4.63, d (5.4) |
| CH$_2$COCH$_3$ | 3.21, overlap | 47.5 t | 3.42, m | 47.1 t | 3.35, m | 47.5 t |
|      | 2.71, m | 2.71, m | 2.64, m |
| CH$_2$COCH$_3$ | 208.1 s | 207.8 s | 208.2 s |
| CH$_2$COCH$_3$ | 214.2, s | 31.2 q | 31.0 q | 31.2 q |
| 1'   | 6.56, dd (10.2, 2.4) | 125.5 d | 6.55, dd (10.2, 1.8) | 125.5 d | 6.54, dd (10.2, 1.8) | 125.7 d |
| 2'   | 6.06, d (10.2) | 133.6 d | 6.06, d (10.2) | 133.6 d | 6.09, d (10.2) | 133.6 d |
| 3'   | 3.32, m | 76.8 d | 3.93, m | 76.6 d | 4.06, m | 76.6 d |
| 4'   | 2.46, dd (11.4, 5.4) | 41.6 t | 2.44, dd (10.8, 6.0) | 41.6 t | 2.49, dd (10.8, 6.0) | 40.9 t |
|      | 1.79, t (11.4) | 1.79, t (10.8) | 1.77, t (10.8) |
| 5'   | 68.2 s | 68.4 s | 68.3 s |
| 6'   | 142.8 s | 143.2 s | 143.4 s |
| 7'   | 5.58, s | 127.3 d | 5.57, s | 127.4 d | 5.58, s | 127.4 d |
| 8'   | 4.92, s | 66.4 d | 4.90, s | 64.6 d | 4.92, s | 64.5 d |
| 10'  | 3.36, dd (13.2, 4.8) | 50.5 t | 3.42, dd (13.2, 4.0) | 47.0 t | 3.21, m | 43.2 t |
|      | 2.83, overlap | 2.83, dd (13.2, 5.0) | 3.15, m |
| 11'  | 4.78, m | 64.9 d | 4.24, s | 74.7 d | 4.00, s | 74.5 d |
| 12'  | 132.5 s | 129.2 s | 129.2 s |
| 13'  | 133.4 s | 132.6 s | 132.6 s |
| 14'  | 6.74, s | 105.9 d | 6.78, s | 106.0 d | 6.75, s | 105.8 d |
| 15'  | 147.3 s | 147.3 s | 148.4 s |
| 16'  | 147.2 s | 147.3 s | 147.3 s |
| 17'  | 7.08, s | 106.8 d | 6.92, s | 108.1 d | 7.37, s | 110.3 d |
| OCH$_3$H$_2$O | 5.93, s | 101.7 t | 5.95, s | 101.8 t | 5.95, s | 101.8 t |
| 3'-OCH$_3$ | 5.92, s | 5.94, s | 5.94, s |
| 11'-OCH$_3$ | 5.93, s | 5.94, s | 5.94, s |

$^1$H NMR recorded at 600 MHz, $^{13}$C NMR recorded at 150 MHz
The molecular formula of 7 was determined to be C$_{21}$H$_{25}$NO$_{5}$ from the HRESIMS m/z at 394.1626 [M+Na]$^+$. Its $^1$H NMR spectrum showed two aromatic singlet protons (δ$_H$ 6.84 and 6.76), three conjugate olefin signals (δ$_H$ 6.60, 6.10 and 5.67), and four methoxy groups (δ$_H$ 3.28, 3.70, 3.78 and 3.97). The $^{13}$C NMR spectrum of 7 showed three methylenes (δ$_C$ 24.6, 43.3, 44.4), two methines (δ$_C$ 76.9 and 70.5) and a carbonyl (δ$_C$ 172.1). These data suggested 7 might be a carboxymethyl derivative of erysotrine [17]. The HMBC correlations from δ$_H$ 3.78 (OCH$_3$) and δ$_H$ 4.14 (CH$_2$) to δ$_C$ 172.1 (C=O) assigned the carboxymethyl at C-8. The molecular formula of 8 was determined to be C$_{20}$H$_{21}$NO$_{5}$ from the HRESIMS m/z at 372.1444 [M+H]$^+$. The $^1$H and $^{13}$C NMR signal of δ$_H$ 2.13 (CH$_3$) and δ$_C$ 207.7 (CH$_2$COOH) in 5 were changed to δ$_H$ 3.61 (OCH$_3$) and δ$_C$ 172.5 (CH$_2$COOH) in 9, respectively. The remaining NMR data were almost identical to those of 5. Thus, the structure of 9 was determined to be 8-acetamethoxyerysotrine and 8-acetamethoxyerythranine, respectively.

Alkaloid 9 showed molecular ion peaks at m/z 370.1652 [M+H]$^+$, suggesting the molecular formulae C$_{21}$H$_{24}$NO$_{5}$. In comparing with compound 5, the $^1$H and $^{13}$C NMR signal of δ$_H$ 2.13 (CH$_3$) and δ$_C$ 207.7 (CH$_2$COOH) in 5 were changed to δ$_H$ 3.61 (OCH$_3$) and δ$_C$ 172.5 (CH$_2$COOH) in 9, respectively. The remaining NMR data were almost identical to those of 5. Thus, the structure of 9 was determined to be 8-acetamethoxyerythraline.

The molecular formulas of compounds 10 and 11 were deduced to be C$_{21}$H$_{23}$NO$_{6}$ and C$_{22}$H$_{25}$NO$_{6}$ from the HRESIMS m/z at 386.1599 [M+H]$^+$ and 400.1758 [M+H]$^+$, respectively. The $^1$H and $^{13}$C NMR data of both 10 and 11 are very similar to those of 9 except that the methylene signal was replaced by an oxymethine signal at the C-11 position. Further, in the $^{13}$C NMR, the signal for C-11 appeared at 64.8 and 74.7 ppm for compounds 10 and 11, similar to that of 4 and 6, respectively. Thus, 10 was identified as 8-acetamethoxyerysotrine. 11 had an extra methoxy and was identified as 8-acetamethoxy-10β-methoxyerythraline.

The molecular formula of 12 was established as C$_{22}$H$_{27}$NO$_{5}$ based on the HRESIMS m/z = 386.1964 [M+H]$^+$. From the $^1$H and $^{13}$C NMR data, the structure of 12 was very similar to 9 except for the replacement of methylenedioxy group by two methoxys at C-15 and C-16. This was confirmed from the HMBC and HSQC spectra. Alkaloid 12 was thus identified as 8-acetamethoxyerysotrine.

The HRESIMS m/z at 394.1628 [M+Na]$^+$ of 13 assigned the molecular formula to be C$_{21}$H$_{25}$NO$_{7}$. 58 mass units higher than that of erysotrine. Its $^{13}$C NMR spectrum gave an additional methylene (δ$_C$ 34.9) and a carbonyl (δ$_C$ 172.3) signals, indicating the existence of an acetyl group. The HMBC correlations from δ$_H$ 2.51 (CH$_3$CO) and δ$_H$ 4.14 (H-8) to δ$_C$ 172.3 (C=O) suggested that the acetyl group was located at C-8. Accordingly, the structure of 13 was determined to be 8-acetylerysotrine. The molecular formula of 14 was determined to be C$_{20}$H$_{21}$NO$_{5}$ by the HRESIMS m/z at 378.1313 [M+Na]$^+$. The 1D NMR spectrum gave signals similar to that of erythraline expect for the replacement of a methylene by an acetyl group (δ$_C$ 35.4 and δ$_C$ 172.3). In the HMBC spectrum, the correlations from δ$_H$ 4.07 (H-8) to δ$_C$ 172.3 (COOH) and δ$_H$ 2.74 (CH$_2$COOH) to δ$_C$ 65.4 (C-8) and δ$_C$ 172.3 (COOH) confirmed that 14 was an 8-acetyl derivative of erythraline.

The configurations of H-8 for compound 1–14 were determined to be β based on ROESY experiments with correlations of H-3β/H-4$_{eq}$, H-4$_{eq}$/H-10$_{ax}$ and H-10$_{eq}$/H-8 (Fig. 2). Further, together with 5S-configuration in all Erythrina alkaloids [18], so absolute configuration of alkaloids 1–14 could be determined.

Since N-containing compounds were main candidates of anticancer and hypoglycemic drugs, so alkaloids 1–14 were evaluated for their cytotoxicity against human A-549 lung cancer, SGC-7901 gastric cancer, and HeLa cell lines using the MTT method. In addition, their hypoglycemic activity on 3T3-L1 myoblasts cell were screened. Unfortunately, none of them showed positive activity. Alkaloids 1–14 possessed acetonyl, acetyl methyl, acetate, or methyl formate groups, which indicated they were artificial products. Without considering the artificial units, these alkaloids are known. Duing the extraction and isolation, methanol, acetone, petroleum ether, especial ethyl acetate, were used as solvents. Accordingly, acetone and residual of acetic acid, methyl acetate and methyl formate in above solvents would become reaction reagents. Alkaloids 1–6 and 9–14 were formed firstly through an iminium immediate by oxidation, then by nucleophilic attack from carbanion of acetone, acetic acid, and methyl acetate in base condition. On the other hand, the iminium immediate could be tautomerized to inmine and

![Fig. 2 Selected NOE interaction of alkaloid 9](image-url)
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3 Experimental Section

3.1 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 400 MHz spectrometers with SiMe$_4$ as an internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. MS data were recorded on an UPLC-IT-TOF MS. 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, Fuji Silysia 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 1525 pumps coupled with analytical 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.

3.2 Plant Material

Flowers of *E. variegata* Linn and *E. crista-galli* Linn were collected in February and April, respectively, 2014 in Simao of Yunnan Province, People’s Republic of China. Leaves and flowers of *Erythrina arborescens* Roxb. Hort. Beng were collected in October 2014 in Jianshui of Yunnan Province. These plant samples were identified by Dr. Chun-Xia Zeng. The voucher specimens (Cai20140207, Cai20140407, Cai20141003 and Cai20141004) have been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, the Chinese Academy of Sciences.

3.3 Extraction and Isolation

The dried and powdered flowers of *E. variegata* (10.0 kg) were extracted with 90% MeOH (25 L) for three times. The extracts were concentrated under reduced pressure, and then dissolved in 2% acetic acid to adjust pH to 2–3 and then partitioned twice with EtOAc. The aqueous layers were basified with NH$_3$·H$_2$O to adjust pH to 8–9 and then extracted with EtOAc to give a crude alkaloid fraction (110 g). The crude alkaloid was subjected to column chromatography (CC) over silica gel with gradient CHCl$_3$-Acetone (1:0 to 1:1) to afford seven fractions (Fr. I–Fr. VII). Fr. I (6.1 g) was divided into 2 subfractions (Fr. I-1–Fr. I-2) by using RP-MPLC eluting...
with MeOH–H2O (50–100%). Fr. I-2 was isolated by preparative C18 HPLC column with a gradient of MeOH–H2O (60:40–70:30, v/v) to obtain 9 (35 mg) and 11 (41 mg). Fr. II (4.5 g) was separated using C18 MPLC column with a gradient of MeOH–H2O (40:60–90:40, v/v) to afford 10 (35 mg) and 8 (13 mg). Fr. IV (8.5 g) was fractionated by C18 MPLC column with a gradient of MeOH–H2O (10:90–90:10, v/v) to give six subfractions (Fr. IV-1–Fr. IV-6). Fr. IV-2 was subjected to a preparative C18 HPLC column with a gradient of MeOH–H2O (50:50–60:40, v/v) to afford 6 (20 mg) and 5 (32 mg). Fr. IV-4 was subjected to a preparative C18 HPLC column with a gradient of MeOH–H2O (70:30–80:20, v/v) to give 1 (5 mg). Fr. V (12 g) was chromatographed on a C18 MPLC column eluted with a gradient of MeOH–H2O (10:80–100:0, v/v) to give six subfractions (Fr. V-1–Fr. V-6). 2 (29 mg) and 3 (21 mg) was obtained from Fr.V-5 using a preparative C18 HPLC column with a gradient of MeOH–H2O (65:35–25:75, v/v).

Flowers of E. crista-galli (11 kg) were powdered and extracted with 90% MeOH (25 L) for three times. The extract was concentrated in vacuo to give a brown residue. The crude alkaloid (90 g) were obtained using the same acid–base treatment method described above, and then subjected to column chromatography (CC) over silica gel and eluted with gradient CHCl3-Acetone (1:0 to 1:1) to afford four fractions (Fr. I–Fr. IV). Fr. I (12.1 g) was further chromatographed on a C18 MPLC column eluted with a gradient of MeOH–H2O (50:50–100:0, v/v) to give the two subfractions (Fr. I-1–Fr. I-2). Alkaloid 12 (51 mg) was obtained from Fr.I-1 using a column chromatography (CC) over silica gel and eluted with petroleum ether-acetone (4:1).

Crude alkaloid extract (85.2 g) and (62.5 g) were obtained from the leaves (15.8 kg) and flowers (6.5 kg) of Erythrina arborescens, respectively. The crude alkaloid of leaves was divided into nine fractions (Fr. I–Fr. IX). Fr. V (7.1 g) was fractionated by C18 MPLC column with a gradient of MeOH–H2O (20:80–80:20, v/v) to give three subfractions (Fr. V-I–Fr. V-3). Fr. V-3 was subjected to a preparative C18 HPLC column with a gradient of MeOH–H2O–H2O (60:40–70:30, v/v) to afford 4 (1 mg) and 14 (5 mg). The crude alkaloid of flowers was divided into seven fractions (Fr. I–Fr. VII). 7 was obtained from Fr. II by C8 MPLC column with a gradient of MeOH–H2O–H2O (40:60–100:0, v/v) and then purified by preparative C18 HPLC column with a gradient MeOH–H2O (50:50–60:40, v/v). Fr. IV (4.5 g) was chromatographed on a C18 MPLC column eluted with a gradient of MeOH–H2O–H2O (20:80–70:30, v/v) to give four subfractions (Fr. IV-1–Fr. IV-4). Fr. IV-3 was further purified by a preparative C18 HPLC column with a gradient of MeCN–H2O (25:75–35:65, v/v) to afford 13 (20 mg).

8α-(2-oxopropyl)-erythryvarine A (1): white powder; [α]D20 121.2 (c 0.2, MeOH); UV (MeOH) λmax (log ε) 204 (4.26), 239 (3.88) and 290 (3.46) nm; IR (KBr) νmax 3441, 2924, 1640, 1503, 1480, 1226, 1100, 1041 cm−1; 1H (60 MHz) and 13C NMR (150 MHz) data (acetone-d6), see Table 1; positive HRESIMS m/z 681.2811 [M+H]+ (calcd. for C39H41N2O9, 681.2110).

8α-(2-oxopropyl)-11′-O-methyl-erythryvarine A (2): white powder; [α]D20 14.1 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 203 (4.12), 238 (3.66) and 289 (3.43) nm; IR (KBr) νmax 3441, 1639, 1490, 1234, 1101, 1040 cm−1; 1H (600 MHz) and 13C NMR (150 MHz) data (acetone-d6), see Table 1; positive HRESIMS m/z 695.2968 [M+H]+ (calcd. for C40H43N2O9, 695.2969).

8α-(2-oxopropyl)-11′-epi-O-methyl-erythryvarine A (3): white powder; [α]D20 +165.2 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 204 (4.22), 238 (3.88) and 289 (3.46) nm; IR (KBr) νmax 3441, 2924, 1629, 1503, 1482, 1234, 1100, 1040 cm−1; 1H (600 MHz) and 13C NMR (150 MHz) data (acetone-d6), see Table 1; positive HRESIMS m/z 695.2970 [M+H]+ (calcd. for C40H43N2O9, 695.2969).

8α-(2-oxopropyl)-erythrine (4): white powder; [α]D20 +133.0 (c 0.2, MeOH); UV (MeOH) λmax (log ε) 204 (4.71), 239 (3.63) and 289 (3.32) nm; IR (KBr) νmax 2930, 1630, 1503, 1489 cm−1; 1H (600 MHz) and 13C NMR (150 MHz) data (acetone-d6), see Tables 2 and 4; positive HRESIMS m/z 370.1651 [M+H]+ (calcd. for C21H23NO5, 370.1652).

8α-(2-oxopropyl)-erythraline (5): white powder; [α]D20 +80.5 (c 0.1, MeOH); UV(MeOH) λmax (log ε) 201 (4.11), 238 (2.37), 289(1.29) nm; IR (KBr) νmax 2930, 1630, 1503, 1489 cm−1; 1H (600 MHz) and 13C NMR (150 MHz) data (acetone-d6), see Tables 2 and 4; positive HRESIMS m/z 354.1709 [M+H]+ (calcd. for C21H23NO5, 354.1707).

8α-(2-oxopropyl)-11′-methoxy-erythraline (6): white powder; [α]D20 +95.3 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 204 (4.76), 238 (3.74) and 288 (3.48) nm; IR (KBr) νmax 1631, 1504, 1483 cm−1; 1H (600 MHz) and 13C NMR (150 MHz) data (acetone-d6), see Tables 2 and 4; positive HRESIMS m/z 384.1806 [M+H]+ (calcd. for C22H25NO6, 384.1805).

8α-carboxethoxyerysotrine (7): white powder; [α]D20 +74.0 (c 0.21, CH3OH); UV (CH3OH) λmax (log ε) 203 (3.79), 225 (3.45) and 277 (3.05) nm; 1H (400 Hz) and 13C (125 Hz) NMR data (acetone-d6), Tables 2 and 4; Positive ESIMS m/z 394 [M+Na]+. HRESIMS m/z. 394.1626 [M+Na]+; (calcd. for C23H25NO2Na, 394.1625).

8α-carboxethoxyerythrine (8): white powder; [α]D20 +118.3 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 205 (4.69), 239 (3.79) and 289 (3.51) nm; IR (KBr) νmax 2923, 1630, 1503, 1488 cm−1; 1H (600 MHz) and 13C NMR (150 MHz) data (acetone-d6), see Tables 2 and 4; positive HRESIMS m/z 372.1444 [M+H]+ (calcd. for C20H22NO6, 372.1443).

8α-acetatethoxyerythrine (9): white powder; [α]D20 +171 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 204
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(4.78), 239 (3.38) and 290 (3.52) nm; IR (KBr) \( \nu \) max 1628, 1501, 1489 cm\(^{-1} \); 1H (600 MHz) and 13C NMR (150 MHz) data (acetone-\( d_6 \)), see Tables 3 and 4; positive HRESIMS m/z 370.1652 [M+H]\(^+\) (calcd. for C\(_{21}\)H\(_{24}\)NO\(_5\), 370.1653).

*8α-acetylamethoxyerythrinine* (10): white powder; \( [\alpha]_D^{20} + 89.4 \) (c 0.1, MeOH); UV (MeOH) \( \lambda_{max} (log e) \) 204 (4.65), 238 (3.68) and 290 (3.33) nm; IR (KBr) \( \nu_{max} \) 2924, 1628, 1488 cm\(^{-1} \); 1H (600 MHz) and 13C NMR (150 MHz) data (acetone-\( d_6 \)), see Tables 3 and 4; positive HRESIMS m/z 386.1599 [M+H]\(^+\) (calcd. for C\(_{21}\)H\(_{24}\)NO\(_5\), 386.1598).

*8α-acetylamethoxy-11β-methoxyerythraline* (11): white powder; \( [\alpha]_D^{20} + 154.2 \) (c 0.2, MeOH); UV (MeOH) \( \lambda_{max} (log e) \) 204 (4.62), 238 (3.74) and 289 (3.41) nm; IR (KBr) \( \nu_{max} \) 1630, 1503, 1484 cm\(^{-1} \); 1H (600 MHz) and 13C NMR (150 MHz) data (acetone-\( d_6 \)), see Tables 3 and 4; positive HRESIMS m/z 400.1758 [M+H]\(^+\) (calcd. for C\(_{22}\)H\(_{26}\)NO\(_6\), 400.1757).

*8α-acetylamethoxyerysotrine* (12): white powder; \( [\alpha]_D^{20} + 113.3 \) (c 0.1, MeOH); UV (MeOH) \( \lambda_{max} (log e) \) 204 (4.72), 239 (3.66) and 288 (3.51) nm; IR (KBr) \( \nu_{max} \) 1628, 1503, 1488 cm\(^{-1} \); 1H (600 MHz) and 13C NMR (150 MHz) data (acetone-\( d_6 \)), see Tables 3 and 4; positive HRESIMS m/z 386.1964 [M+H]\(^+\) (calcd. for C\(_{22}\)H\(_{28}\)NO\(_5\), 386.1965).

Table 2

| Entry | \( \delta_H (4)^{a} \) | \( \delta_H (5)^{a} \) | \( \delta_H (6)^{a} \) | \( \delta_H (7)^{b} \) | \( \delta_H (8)^{a} \) |
|-------|------------------|------------------|------------------|------------------|------------------|
| 1     | 6.55, dd (10.2, 2.4) | 6.72, dd (10.2, 2.4) | 6.55, dd (10.2, 1.8) | 6.60 dd (10.2, 1.9) | 6.59, dd (10.2, 2.4) |
| 2     | 6.01, d (10.2) | 6.68, d (10.2) | 6.03, d (10.2) | 6.10, d (10.2) | 6.07, d (10.2) |
| 3     | 3.79, m | 3.85, m | 3.92, m | 3.97, m | 3.85, m |
| 4     | 2.38, dd (11.4, 5.4) | 2.48, dd (11.4, 5.4,6) | 2.46, dd (11.4, 5.4) | 2.53, dd (11.2, 5.7) | 2.45, dd (11.4, 5.4) |
| 7     | 5.67, s | 5.63, s | 5.64, s | 5.67, s | 5.71, s |
| 8     | 4.20, m | 3.98, m | 4.33, m | 4.33, s | 4.68, s |
| 10    | 3.48, dd (13.8, 4.8) | 3.38, m | 3.39, dd (13.8, 4.2) | 3.49, m | 3.57, dd (13.2, 4.8) |
| 11    | 4.75, m | 2.77 (overlap) | 4.18, t (4.2) | 2.93, m | 4.77, m |
| 14    | 6.70, s | 6.72, s | 6.74, s | 6.84, s | 6.73, s |
| 17    | 7.05, s | 6.68, s | 6.89, s | 6.76, s | 7.04, s |
| 3-OCH\(_3\) | 3.25, s | 3.28, s | 3.28, s | 3.28, s | 3.23, s |
| 11-OCH\(_3\) | 3.55, s | 3.55, s | 3.55, s | 3.55, s | 3.55, s |
| 11-OH | 4.72, d (4.8) | 3.70, s | 3.97, s | 3.70, s | 3.70, s |
| 15-OCH\(_3\) | 6.01, s | 5.92, s | 5.96, s | 5.95, s | 5.94, s |
| 16-OCH\(_3\) | 5.99, s | 5.90, s | 5.94, s | 5.94, s | 5.94, s |
| OCH\(_2\)O | 2.90, dd (15.6, 4.8) | 2.85 (overlap) | 2.94, overlap | 2.94, overlap | 2.94, overlap |
| CH\(_2\)COCH\(_3\) | 2.55, dd (15.6, 4.8) | 2.58, dd (16.8, 8.0) | 2.55, dd (15.6, 9.0) | 2.55, dd (15.6, 9.0) | 2.55, dd (15.6, 9.0) |
| CH\(_2\)COCH\(_3\) | 2.13, s | 2.13, s | 2.12, s | 2.12, s | 2.12, s |
| COOCH\(_3\) | 3.78, s | 3.78, s | 3.78, s | 3.78, s | 3.78, s |

\(^{a}\)H NMR recorded in 600 MHz

\(^{b}\) H NMR recorded in 400 MHz

3.4 Cytotoxicity

The human A-549 lung cancer, SGC-7901 gastric cancer, and HeLa cell lines were used in the cytotoxic assay. These cells were grown in DMEM media (HyClone, USA) supplemented with 10% fetal bovine serum (HyClone, USA) at 37 °C in 5% CO\(_2\). The cytotoxicity of all alkaloids was
determined based on the MTT method in 96-well microplates. In short, 100 µL adherent cells were seeded into each well and incubated for 12 h before the addition of the test alkaloids/drug. At the same time, the suspended cells were seeded at an initial density of 1 × 10⁵ cells/mL just before the addition of the alkaloids/drug. Each tumor cell line was exposed to a test compound at concentration 20 μM in DMSO in triplicate for 48 h, with camptothecin as the positive control. After treatment, cell viability was assessed.

3.5 Hypoglycemic Activity

3T3-L1 myoblasts cells were purchased from American Type Culture Collection (Manassas, VA). Cells were maintained in DMEM supplemented with 10% FBS or CS (for 3T3-L1 cells), 100 units/ml penicillin and 100 mg/ml streptomycin in 10 cm diameter dishes in a humidified atmosphere of 95% air and 5% CO₂ at 37 °C. Cells were maintained in continuous passages by trypsinization of subconfluent cultures and fed fresh medium every 48 h. For differentiation, L6 myoblasts were transferred to DMEM with 2% FCS in tissue culture plates for 5-6 days, 3T3-L1 cells were exposed to 0.5 mM IBMX, 1 mM dexamethasone, 1 mM rosiglitazone and 1 mg/mL insulin for 3 days, and 1 mg/mL insulin for the other day. For glucose uptake assay, cells were serum starved for 4 h in 96-well plates, followed by incubated with insulin and alkaloids 1–14 for 24 h. Finally, the supernatants of cultured cells were collected and subjected to glucose assay using a commercially kit. The quantified values were normalized based on the results of the MTS assay.

### Table 3 ¹H NMR spectroscopic data for 9–14 in acetone-d₆ (δ in ppm, J in Hz)

| Entry | δ₁H(9) a | δ₁H(10) a | δ₁H(11) a | δ₁H(12) b | δ₁H(13) b | δ₁H(14) b |
|-------|----------|----------|----------|----------|----------|----------|
| 1     | 6.54, dd 10.2, 2.4 | 6.55, dd 10.2, 2.4 | 6.56, dd 10.2, 1.8 | 6.57, dd 10.2, 2.4 | 6.60, dd 10.2 | 6.58, dd 10.2, 2.2 |
| 2     | 6.00, d 10.2 | 5.99, d 10.2 | 6.04, d 10.2 | 6.05, d 10.2 | 6.14, d 10.20 | 6.11, d 10.2 |
| 3     | 3.8, m | 3.77, m | 3.91, m | 4.01, m | 4.09, m | 3.92, m |
| 4     | 2.48, dd 11.4, 5.4 | 2.38, overlap | 2.46, dd 11.4, 5.4 | 2.50, m | 2.63, dd 10.9, 5.7 | 2.66, dd 11.4, 5.5 |
| 7     | 5.66, s | 5.70, s | 5.69, s | 5.64, s | 5.68, s | 5.72, s |
| 8     | 3.94, m | 4.16, m | 4.31, m | 4.05, m | 4.14, m | 4.07, m |
| 10    | 3.40, m | 3.50, dd 12.0, 5.4 | 3.39, dd 13.8, 4.2 | 3.37, m | 3.49, m | 3.53, m |
| 11    | 2.84, overlap | 4.77, m | 4.19, t 4.2 | 2.96, m | 3.03, m | 2.98, m |
| 12    | 2.65, overlap | 2.57, m | 2.72, overlap | 2.84, m | 6.70, s | 6.69, s |
| 14    | 6.70, s | 6.69, s | 6.90, s | 6.83, s | 6.84, s | 6.73, s |
| 15    | 6.68, s | 7.05, s | 6.74, s | 6.81, s | 6.78, s | 6.74, s |
| 16    | 3.25, s | 3.23, s | 3.29, s | 3.28, s | 3.31, s | 3.30, s |
| 11-OCH₃ | 3.25, s | 3.55, s | 3.55, s | 3.55, s | 3.55, s | 3.55, s |
| 11-OH | 4.60, d 5.4 | 3.78, s | 3.78, s | 3.78, s | 3.78, s | 3.78, s |
| 15-OCH₃ | 3.67, s | 3.79, s | 3.67, s | 3.79, s | 3.67, s | 3.79, s |
| 16-OCH₃ | 5.91, s | 5.95, s | 5.96, s | 5.96, s | 5.96, s | 5.96, s |
| OCH₂O | 5.89, s | 5.94, s | 5.94, s | 5.94, s | 5.94, s | 5.94, s |
| CH₂COO⁻ | 2.68, dd 15.0, 4.8 | 2.73, dd 15.0, 4.8 | 2.81, dd 15.0, 4.2 | 2.72, dd 15.0, 4.8 | 2.76, overlap | 2.74, dd 16.8, 5.8 |
| CH₂COOCH₃ | 2.39, dd 15.0, 7.8 | 2.36, overlap | 2.36, dd 15.0, 6.0 | 2.41, dd 15.0, 7.8 | 2.51, dd 16.5,2.8 | 2.51, dd 16.8, 1.8 |

a¹H NMR recorded in 600 MHz

b¹H NMR recorded in 400 MHz
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Compliance with Ethical Standards

Conflicts of interest The authors declare no conflict of interest.

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Table 4 13C NMR spectroscopic data from 4 to 14 in acetone-d6 (δ in ppm)

| Entry | δC (4)a | δC (5)a | δC (6)a | δC (7)b | δC (8)b | δC (9)a | δC (10)a | δC (11)a | δC (12)a | δC (13)b | δC (14)a |
|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 1     | 125.4 d 125.6 d 125.7 d 125.3 d 125.4 d 125.3 d 125.5 d 125.6 d 125.1 d 125.0 d |
| 2     | 133.1 d 133.1 d 134.0 d 134.2 d 133.1 d 133.2 d 133.1 d 133.2 d 134.1 d 134.1 d |
| 3     | 76.9 d 77.0 d 76.8 d 76.9 d 76.8 d 76.8 d 76.9 d 76.7 d 77.0 d 76.6 d 76.5 d |
| 4     | 43.3 t 43.8 t 42.7 t 43.3 t 43.1 t 43.8 t 43.6 t 42.7 t 43.4 t 42.3 t 42.7 t |
| 5     | 69.3 s 69.3 s 68.6 s 69.4 s 69.7 s 69.6 s 69.7 s 68.9 s 68.9 s 69.1 s 69.7 s |
| 6     | 142.3 s 142.6 s 142.4 s 144.4 s 142.7 s 142.4 s 142.4 s 142.9 s 143.1 s 143.0 s |
| 7     | 127.6 d 127.6 d 127.9 d 121.7 d 122.4 d 126.9 d 127.1 d 127.3 d 126.7 d 125.2 d 125.6 d |
| 8     | 67.7 d 65.1 d 65.8 d 70.5 d 73.2 d 66.1 d 68.5 d 66.4 d 64.4 d 63.9 d 65.4 d |
| 10    | 52.6 t 43.9 t 45.2 t 44.4 t 53.1 t 44.2 t 53.0 t 45.4 t 42.6 t 40.8 t 42.3 t |
| 11    | 64.7 d 26.0 t 74.8 d 24.6 t 65.1 d 26.0 t 64.8 t 74.7 d 24.6 t 24.1 t 25.5 t |
| 12    | 132.2 s 129.4 s 130.1 s 128.0 s 133.3 s 129.4 s 133.5 s 130.0 s 127.7 s 126.8 s 128.7 s |
| 13    | 133.4 s 134.0 s 133.0 s 132.2 s 131.9 s 133.9 s 132.2 s 132.9 s 132.7 s 130.9 s 132.2 s |
| 14    | 105.9 d 106.5 d 105.9 d 110.6 d 105.8 d 106.4 d 105.8 d 105.7 d 110.6 d 110.4 d 106.4 d |
| 15    | 147.3 s 146.7 s 148.0 s 148.2 s 147.6 s 146.6 s 147.3 s 147.8 s 148.9 s 148.5 s 147.0 s |
| 16    | 147.2 s 147.0 s 147.2 s 149.1 s 147.5 s 146.9 s 147.3 s 147.1 s 148.1 s 149.4 s 147.5 s |
| 17    | 107.4 d 109.4 d 109.0 d 113.1 d 108.1 d 109.3 d 107.2 d 108.8 d 113.0 d 113.1 d 109.5 d |
| 3-OCH3 | 56.2 q 56.3 q 56.3 q 56.0 q 56.3 q 56.1 q 56.2 q 56.0 q 55.9 q 56.4 q 56.4 q |
| 11-OCH3 | 57.9 q 57.7 q 56.0 q 56.3 q |
| 15-OCH3 | 56.1 q 56.1 q 56.3 q 56.1 q |
| 16-OCH3 | 56.1 q 55.9 q |
| OCH2O | 101.7 t 101.7 t 101.9 t 101.9 t 101.5 t 101.7 t 101.7 t 101.9 t 34.9 t 35.4 t |
| CH3COCH3 | 47.8 t 48.1 t 47.4 t 48.1 t |
| CH2COCH3 | 207.9 s 207.7 s 207.7 s 207.7 s |
| CH2COCH3 | 30.8 q 30.9 q 30.8 q 30.8 q |
| (CH2)COOCH3 | 39.5 t 39.3 t 38.8 t 39.3 t |
| (CH2)COOCH3 | 172.1 s 172.2 s 172.5 s 172.4 s 172.3 s 172.9 s |
| (CH2)COOCH3 | 52.0 q 52.1 q 51.5 q 51.5 q 51.5 q 51.6 q |

a13C NMR recorded in 150 MHz
b13C NMR recorded in 125 MHz
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