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Cytotoxic alkaloids from the fruits and seeds of *Alangium salviifolium* (L.f.) Wangerin

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ABSTRACT

One new cepheline-type alkaloid 8-hydroxyl-cepheline (**1**) and one new natural product $\Delta^{1',2'}$-deoxytubulosine (**2**), together with 9 known alkaloids have been isolated from the fruits and seeds of *Alangium salviifolium* (L.f.) Wangerin. The structures of the new alkaloids were elucidated by means of extensive spectroscopic analysis (2D NMR, HRESIMS). All alkaloids were evaluated for their cytotoxic activity against three human cancer cell lines, A-549, Hela and SKOV-3. Compounds **1**-**4**, **6**, **8** and **9** exhibited significant inhibitory effects against three human cancer cells with IC_{50} values (0.1–14 μM) comparable to those of cisplatin.

1. Introduction

Alangium salviifolium (L.f.) Wangerin (Cornaceae) is a small deciduous tree or shrub, of which the fruits are produced. This plant mainly grows in the wild throughout the hotter parts of China, India, Malaysia and Phillippines (Anonymous, 1985). In Guangdong, Guangxi and Hainan provinces of China, this plant was called “Ge-She-Luo” by local people, meaning that the fruit of *A. salviifolium* (L.f.) Wangerin can cause damage to tongue and mouth when eaten too much (Chen et al., 2012). We thought those components which can cause damage to human tissue cells may be cytotoxicity and have potential in the treatment of human tumors. In our continuous search for structurally and biogenetically cytotoxic alkaloids from toxic plants (Chen and Zheng, 1987; Qu et al., 2013; Zhou et al., 2018), which can produce new chemical entities of new anti-tumor drugs (Wani et al., 1971; Gorman et al., 1959; Recher et al., 1972), one new cepheline-type alkaloid, one new natural product and 9 known alkaloids were isolated from the fruits and seeds of *A. salviifolium* (L.f.) Wangerin. Herein, we report the isolation, structural elucidation, and the cytotoxic activity of these eleven alkaloids.

2. Results and discussions

The concentrated ethanol extract of *A. salviifolium* (L.f.) Wangerin was suspended in water and successively treated with acidic water and then extracted with EtOAc to remove the nonalkaloids. The acidic aqueous phase was basified with Na_2CO_3 and subsequently extracted with $CHCl_3$ to afford the crude alkaloids. The crude alkaloids were subjected to various chromatographic separations to afford eleven alkaloids (**1**-**11**), including one new cephaeline-type alkaloid (**1**) and one new natural product (**2**). Their structures were identified by spectroscopic analysis including 1D, 2D NMR and HRESIMS data. Nine known compounds were identified as cepheline (**3**) (Teshima et al., 1989), isocephaeline (**4**) (Itoh et al., 2000), 2′-N-(1″-Deoxy-1″-β-D-fructopyranosyl)cephaeline (**5**) (Itoh et al., 1999), deoxytubulosine (**6**) (Monteiro et al., 1965), deoxyisotubulosine (**7**) (Brown et al., 1979), alangimarckine (**8**) (Battersby et al., 1966), tubulosine (**9**) (Ma et al., 1990), compound **10** (Itoh et al., 1998), alangiside (**11**) (Itoh et al., 1991), by comparison of their NMR and MS spectral data with those reported in the literature.

Compound **1** was isolated as a yellow amorphous powder and showed a positive response with Dragendorff's reagent on TLC. The molecular formula $C_{28}H_{38}N_2O_5$ was deduced from HR-ESI-MS (*m/z* = 483.2852, calcd for $[M+H]^+$ 483.2859) and ^{13}C NMR spectroscopic data, with 11 degrees of unsaturation. Its UV spectrum displayed UV-absorption at 205 and 283 nm typical of benzoquinolizidine chromophores. The IR spectrum showed the existence of NH (3379 cm^{-1}) and methyl (2931 cm^{-1}) groups. Its ^1H NMR spectrum exhibited signals

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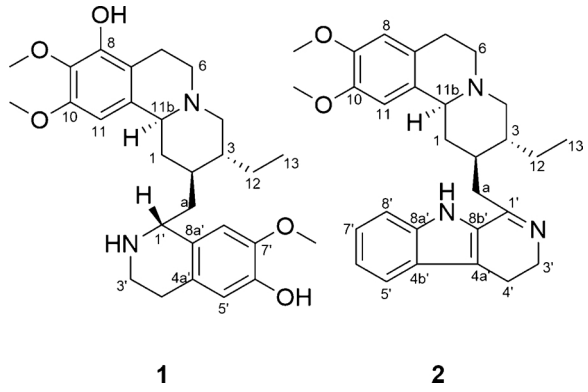

Fig. 1. Structures of compounds **1** and **2**.

for three methoxyl groups at δ 3.86 (3H, s), 3.81 (3H, s) and 3.79 (3H, s), signals for an ethyl group at δ 0.89 (3H, t, J = 7.46 Hz), 1.11 (1H, m) and 1.64 (1H, m) and three aromatic singlets at δ 6.59, 6.49 and 6.38. Its ^{13}C NMR and ^{1}H NMR spectrum showed nine quaternary sp^2 carbons, seven methines including three sp^2 and four sp^3 carbons, eight methylenes and four methyl groups. The chemical shift patterns of compound **1** were characteristic of those found in compound **3**, further verified by analysis of the HSQC, ^{1}H-^{1}H COSY, and HMBC spectra (Fig. 2). The planar structure of **1** is highly similar to that of compound **3** (cepheline) (Teshima et al., 1989) except position C-8. The aromatic methine at δ_C 111.4 (δ_H 6.61, s) in compound **3** was replaced by one aromatic quaternary (δ_C 146.6) in **1**, meaning one hydroxyl at C-8 instead of the proton in **1**, which was further confirmed by comparison of the molecular weight of compounds **1** and **3**. The key HMBC correlation from H-7 to C-8 further confirmed that the hydroxyl is at C-8 (Fig. 2). Thus, the planar structure of compound **1** was determined as shown in Fig. 1 . In the NOESY spectrum, H-11b (δ_H 3.09), H-1α (δ_H 2.58), and H-2 (δ_H 1.62) showed correlations between each other, indicating that H-11b and H-2 were at the same side to the ring system. On the other hand, H-3 (δ_H 1.42) correlated to H-1β (δ_H 1.24) and H-α (δ_H 1.41), thereby confirming that H-11b, H-2 and the ethyl group were at the same side to the ring system. The absolute configurations at C-2, C-3 and C-11b were deduced to be the same as in **3** by the similarity of the ^{1}H and ^{13}C NMR spectral data for **1** with those in **3** (Figs. S15 and S16), together with biogenetic considerations (Itoh et al., 1999). The remaining configuration of C-1′ was suggested to be R by almost the same coupling constant between H-1′ and H-α as in **3** in the ^{1}H NMR spectrum, along with the chemical shifts of C-1, C-2 and C-1′ in ^{13}C NMR spectrum (Fujii et al., 1985), which were further confirmed by the comparison of the optical rotation of **1** ($[\alpha]_D^{20}$ -5.4 (c 0.18, MeOH) with those of cepheline (**3**) ($[\alpha]_D^{20}$ -3.2 (c 0.25, MeOH) and isocephaeline (**4**) ($[\alpha]_D^{20}$ -54.7 (c 0.24, MeOH). Hence, the absolute configuration of 8-hydroxyl-cepheline was determined to be **1** (Fig. 1).

The molecular formula, $C_{27}H_{36}N_2O_5$, for **2** was established by HRESIMS (*m/z* = 458.2802, calcd for $[M+H]^+$ 458.2808). The UV spectrum demonstrated chromophores typical of the benzoquinolizidine and indole. Its ^{1}H NMR spectrum exhibited signals for two methoxyl groups at δ 3.78 (3H, s) and 3.46 (3H, s), signals for an ethyl group at δ 0.91 (3H, t, J = 7.5 Hz), 1.11 (1H, m) and 1.71 (1H, m) and two aromatic singlets at δ 6.50 and 6.33, and a 1,2-disubstituted benzene ring in the indole moiety with signals at δ_H 7.58 (1H, d, J = 8.2 Hz), 7.13 (1H, t, J = 8.2 Hz), 7.24 (1H, t, J = 8.2 Hz) and 7.36 (1H, d, J = 8.2 Hz). Its ^{13}C NMR and ^{1}H NMR spectrum showed nine quaternary sp^2 carbons, ten methines including six sp^2 and four sp^3 carbons, eight methylenes and three methyl groups. The ^{1}H and ^{13}C NMR spectrum of **2** was found to be similar to those reported for deoxytubulosine, except for the differences associated with the presence of one double bond at C-1′ (δ_C 79.5) and N-2′. Furthermore, a close comparison of the ^{1}H and ^{13}C NMR spectrum of **2** with deoxytubulosine (**6**) (Monteiro et al., 1965) and deoxyisotubulosine (**7**) (Brown et al., 1979), led to the conclusion that **2** is $\Delta^{1',2'}$-deoxytubulosine, which has not been reported previously as a natural product (Battersby et al., 1961). In the NOESY spectrum, H-11b, H-1α, and H-2 showed correlations between each other, and H-3 correlated to H-1β and H-α, indicating that H-11b, H-2 and the ethyl group were at the same side to the ring system. Hence, the relative configuration of compound **2** was determined as shown.

In conclusion, eleven alkaloids including one new alkaloid and one new natural product were isolated from fruits and seeds of *A. salviifolium* (L.f.) Wangerin (Fig. 3). Their chemical structures were elucidated on the basis of spectroscopic evidence, and the NMR data of deoxyisotubulosine (**7**) were reported here for the first time (Figs. S21 and S22). All alkaloids were tested against Hela cervical cancer, A-549 human lung adenocarcinoma and SKOV-3 human ovarian carcinoma cell lines. It is noteworthy that compounds **1**-**4**, **6**, **8**, and **9** showed significant inhibitory effects against three human cancer cell lines with IC_{50} values comparable to or even lower than those of cisplatin (Table 2).

3. Experimental

3.1. General

Optical rotations were recorded using a PerkinElmer Model 341 polarimeter. UV spectra were recorded using a Shimadzu UV-1700 spectrophotometer. IR spectra were recorded on KBr discs using a Thermo Nexus470 FT-IR spectrometer. NMR spectra were acquired with a Bruker BIOSPIN AV 400 NMR instrument at 400 MHz (^{1}H) and 100 MHz (^{13}C). HRESIMS spectra were recorded using a ThermoFisher LTQ Orbitrap XL mass instrument. Column chromatography (CC) was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, China) and Sephadex LH-20 (Pharmacia, Sweden) columns.

3.2. Plant material

The fruits and seeds of *A. salviifolium* (L.f.) Wangerin were collected from Hainan province, the People's Republic of China, and identified by Mr. Shi-Man Huang, College of Life Sciences, Hainan Normal

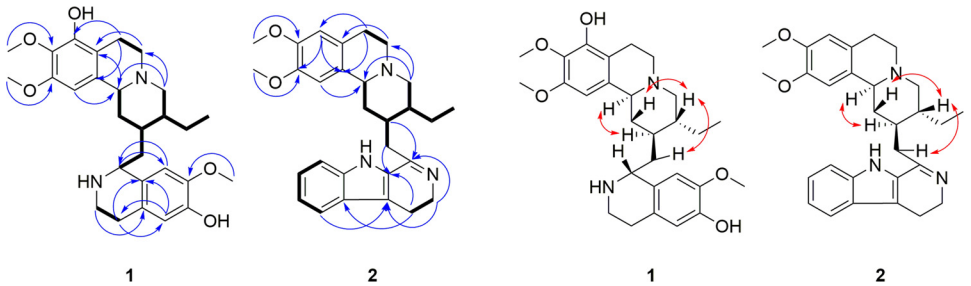

Fig. 2. ^{1}H-^{1}H COSY (left, Bold), selected HMBC (left, Blue Single Arrows), and NOESY (right, Red Double-headed Arrows) correlations of compounds **1** and **2.** (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).