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Article

Protoberberine Isoquinoline Alkaloids from *Arcangelisia gusanlung*

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Abstract: HPLC-DAD-directed isolation and purification of the methanol extract of stems of *Arcangelisia gusanlung* H. S. Lo. led to the isolation of a new protoberberine alkaloid, gusanlung E (**1**), along with fourteen known derivatives **2**–**15**, seven of which were obtained from the genus *Arcangelisia* for the first time. The structures and absolute stereochemistry of these compounds were elucidated on the basis of spectroscopic analyses, including 1D and 2D NMR, mass spectrometry, and CD analyses. Gusanlung E (**1**) expressed weak cytotoxic activity against the SGC 7901 cell line with an IC_{50} value of 85.1 μM.

Keywords: protoberberine alkaloid; *Arcangelisia gusanlung*; gusanlung E

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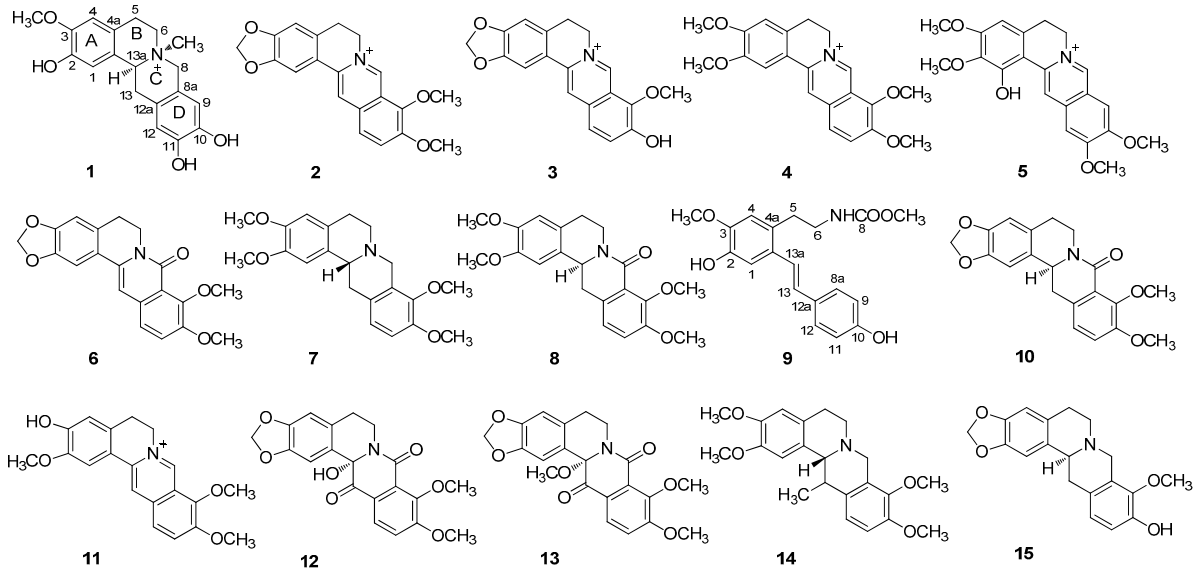
1. Introduction

Arcangelisia gusanlung H. S. Lo (Menispermaceae) is a small shrub widely distributed in the south of China including the provinces of Guangdong, Guangxi, and Hainan. The stems of *A. gusanlung* have been clinically used in Chinese folk medicine as an anti-inflammatory, antipyretic, and detoxication reagent [1]. Previous phytochemical investigations of the plant revealed the presence of a series of protoberberine alkaloids [2–4] and megastigane glycosides [5] in its stems. Protoberberine alkaloids, which belongs to a isoquinoline alkaloid class, are widely distributed in many species of the Berberidaceae, Annonaceae, Fumariaceae, Papaveraceae, Ranunculaceae, Rutaceae, and other plant families, encompassing a diverse class of secondary metabolites with many pharmacologically active members, such as berberine and palmatine [6,7]. Over the last decade, these alkaloids have attracted considerable attention due to their wide range of biochemical and pharmacological actions, which have applications in various therapeutic areas such as cancer, inflammation, diabetes, depression, hypertension, and various infectious areas [8].

In order to further investigate the active components of *A. gusanlung*, HPLC-DAD-directed isolation was carried out on the CH_3OH extract of the stems of *A. gusanlung*. As a result, 15 protoberberine alkaloids including a new one, gusanlung E (**1**), together with fourteen known derivatives **2**–**15**, seven of which were obtained from the genus *Arcangelisia* for the first time (Figure 1). Herein, we report the detailed isolation and structural characterization of these compounds, as well as cytotoxic activity of gusanlung E (**1**).

Figure 1. Structures of compounds **1**–**15**.



2. Results and Discussion

2.1. Structural Characterization

Gusanlung E (**1**) was obtained as yellow crystals, and its molecular formula was determined as $C_{19}H_{22}NO_4$ by HR-ESI-MS at *m/z* 328.1581 $[M]^+$ (calcd. for $C_{19}H_{22}NO_4$: 328.1549), indicating ten degrees of unsaturation. The ^{1}H-, ^{13}C-NMR and HSQC spectroscopic data suggested the presence of

19 carbons. The ^{1}H-NMR spectrum showed four aromatic protons at δ 6.86, 6.71, 6.62 and 6.61, one aromatic methoxyl group at δ 3.87 (3H, s) and an *N*-methyl signal at δ 3.20 (3H, s). The signal at δ 3.26 (2H, m) was assigned as H-5, whereas the signals at 3.49 (1H) and 3.82 (1H) were assigned as germinal protons to H-6. Moreover, signals of a pair of methylene protons and an isolated -CH-CH_2- moiety were found in the aliphatic region. The large coupling constant (15.0 Hz) of a pair of doublets at δ 4.71 and δ 4.52 suggested the existence of germinal protons, which was confirmed by HSQC. This is a typical characteristic of methylene group (C-8) of the protoberberine alkaloids [9]. The signals of an isolated -CH-CH_2- moiety were assigned to C-13a and C-13. In addition, there were three exchangeable protons were observed at δ 9.13 in the proton NMR spectrum of DMSO-d_6. Analysis of the ^{1}H-, ^{13}C-, and HSQC NMR spectroscopic data (Table 1) revealed that there were twelve aromatic carbon signals: four aromatic methylene (δ_C:115.8, 114.5, 114.1, 113.3), eight aromatic quaternary (four oxygenated); four methylene; one methane; one aromatic methoxyl (δ_C 57.6) and one *N*-methyl carbon (δ_C 50.7). According to the above information, the structure of **1** was closely related to the 2,3,10,11-tetrasubstituted-*N*-methyltetrahydroprotoberberine skeleton [10,11]. The complete assignments were accomplished using ^{1}H-^{1}H COSY, HSQC, HMBC and NOESY spectra.

Table 1. ^{1}H- (600 MHz, δ ppm, *J* in Hz), ^{13}C-NMR (150 MHz, δ ppm), COSY and HMBC spectroscopic data for compound **1** in methanol-d_4.

Position	δ_C	δ_H (*J* Hz)	COSY	HMBC
1	114.5, CH	6.71 s		C-3, C-4a, C-13a
1a	125.8, C			
2	147.5, C			
3	150.1, C			
4	113.3, CH	6.83 s		C-2, C-3, C-1a, C-4a, C-5
4a	120.3, C			
5	24.3, CH_2	3.28, 3.23 m	H-6	C-1a, C-4a, C-4
6	53.3, CH_2	3.82, 3.49 m	H-5	C-4a, C-13a, N-CH_3, C-8, C-5
8	65.1, CH_2	4.71, 4.52 d (15)		C-12a, C-8a, C-6, C-9, C-13a, N-CH_3, C-12a
8a	118.0, C			
9	114.1, CH	6.60 s		C-11, C-12a, C-10, C-8a, C-8
10	146.7, C			
11	147.8, C			
12	115.8, CH	6.83 s		C-11, C-10, C-9, C-8a, C-13
12a	122.0, C			
13	35.4, CH_2	3.35 dd (4.8, 19.6) 3.02 dd (10.2, 18.0)	H-13a	C-8a, C-1a, C-12,
13a	67.6, CH	4.66 dd (6.6, 10.2)	H-13	C-12a, C-4a, C-1, C-8, C-13
3-OCH_3	56.7, CH_3	3.87 s		C-3, C-4, C-2
N-CH_3	50.7, CH_3	3.20 s		C-13a, C-6, C-8

Interpretation of the ^{1}H-^{1}H COSY NMR data of **1** confirmed that two isolated proton spin-systems belong to C-5-C-5a and C-13-C-13a units, and the remaining connections were established by analysis of HMBC correlations. The HMBC correlations from -OCH_3 to C-1, C-3, and C-4, whereas correlations from H-1 to C-3, C-13a and C-4a, and from H-4 to C-1a, C-2 and C-5, indicated that A ring possessed 2-OH and 3-OCH_3 substitutions (Figure 2). The result was further confirmed by NOESY spectrum, in which the NOE correlations between 3-OCH_3 and H-4, H-4 and H-5, H-1 and H-13a were observed. In the same way, the cross peaks of H-9 with C-8, C-12a, C-11 and H-12 with

C-10, C-8a, C-13 in the HMBC spectrum suggested dihydroxyl substitutions at C-10 and C-11 in D ring. Moreover, H-9 was correlated with H-8 and H-12 with H-13 in the NOESY spectrum (Figure 3). Therefore, the planar structure of **1** was characterized as 2,10,11-trihydroxy-3-methoxy-*N*-methyltetrahydro-protoberberine.

The relative configuration was determined by a NOESY experiment. The *N*-methyl protons showed a NOE correlation with H-13a (Figure 3). Moreover, the NOE correlation between H-6 and *N*-methyl protons suggested the axial position of H-6. The H-13 signal showed a large coupling constant (12.0 Hz) with the signal of H-13a, indicating that H-13 was at axial position [11]. Meanwhile, the ^{1}H- and ^{13}C-NMR chemical shifts of *N*-methyl group (δ_H 3.20, δ_c 50.7) as well as a NOESY cross peak between the *N*-methyl group and H-13a suggested a B/C-*cis* fused form [12]. Furthermore, the negative value of specific optical rotation and circular dichroism (CD) curve indicated the 7*S*, 13a*S* configurations [13]. Accordingly, the structure of the new compound was elucidated as shown in Figure 1.

Figure 2. ^{1}H-^{1}H COSY and key HMBC correlations of compound **1**.

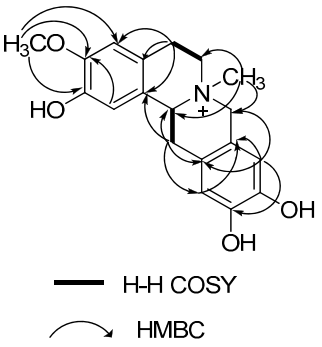
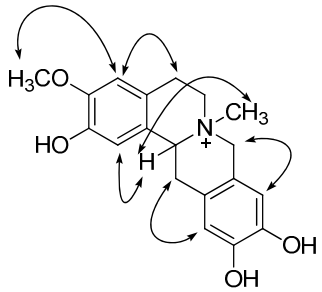


Figure 3. Key NOE of compound **1**.



Compounds **2**–**15** were identified as berberine (**2**) [14], thalifendine (**3**) [15], palmatine (**4**) [16], stephabine (**5**) [17], 8-oxyberbeine (**6**) [18], tetrahydropalmatine (**7**) [19], 8-oxotetrahydroplamatine (**8**) [20], gusanlung C (**9**) [7], gusanlung B (**10**) [6], jatrorrhizine (**11**) [21], 8,13-dioxo-14-hydroxycanadine (**12**) [22], 8,13-dioxo-14-methoxycanadine (**13**) [23,24], corydaline (**14**) [24] and tetrahydrothalifendine (**15**) [25], respectively, by comparison of the ^{1}H- and ^{13}C-NMR data with reported spectroscopic data. Among them, **5**, **7**, **8**, and **12**–**15** were isolated from this plant for the first time.

2.2. Cytotoxic Activities

Gusanlung E (**1**) exhibited weak cytotoxic activity against cell line SGC 7901 with IC_{50} value of 85.1 μM.

3. Experimental Section

3.1. General Experimental Procedures

Optical rotations were recorded on a JASCO DIP-1000 polarimeter (JASCO, Kyoto, Japan). IR spectra were recorded on a Shimadzu FTIR-8400s (Shimadzu, Kyoto, Japan). UV spectra were run on a Shimadzu UV-2550 UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan). CD spectra were measured on a JASCO J-810 spectrometer (JASCO, Kyoto, Japan). 1D and 2 D NMR spectra were measured in methanol-d_4 (δ_H 3.30/δ_C 49.5) on a Bruker Avance III 600 spectrometer (^{1}H: 600 MHz, ^{13}C: 150 MHz) (Munich, Ettlingen, Germany). HRESIMS were obtained using a LTQ Orbitrap XL spectrometer (Thermo Fisher, Bremen, Germany). Analytical HPLC was performed on a Waters 600 with a Waters 2996 photodiode array detector (Waters, Milford, MA, USA). Semipreparative HPLC was performed on a Shimadzu LC-6AD with a Shimadzu SPD-6AD spectrophotometric detector (Shimadzu, Kyoto, Japan).

3.2. Plant Material

The stems of *A. gusanlung* were collected from Wanning City in Hainan Province of The People's Republic of China in August 2008. The sample was identified by Prof. Guobiao Chen from the Institute for Drug Control of Hainan Province. A voucher specimen (No. 200808) was deposited in the herbarium of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing.

3.3. Extraction and Isolation

The air-dried and smashed stems of *A. gusanlung* (18 kg) were extracted with MeOH (3 × 80 L) and afforded a crude extract of 880 g after evaporation of the solvent under vacuum. The extract was suspended in H_2O (2.0 L) and partitioned sequentially with petroleum ether (3 × 3.0 L), EtOAc (3 × 3.0 L), and *n*-BuOH (3 × 3.0 L). The EtOAc extract (40 g) was subjected to chromatography over silica gel (800 g, 100–200 mesh) and eluted with CH_2Cl_2–MeOH to yield six fractions (E1 to E6) on the basis of TLC and HPLC-DAD analyses. Repeated crystallization of fraction E5 (CH_2Cl_2–MeOH) yielded compounds **2** (10 g), **3** (3 g) and **4** (2 g). The *n*-BuOH extract (630 g) was subjected to column chromatography over macroporous resin D101 and eluted successively with EtOH–H_2O (1:9, 3:7, 6:4, and 1:0) to yield four fractions (B1 to B4). Fraction B1 (10 g) was further subjected to column chromatography over macroporous resin AB-8 and eluted successively with EtOH–H_2O (1:9, 2:8, 3:7 and 1:0) to yield five fractions (B1-1 to B1-5). Subfraction B1-3 (1.5 g) was subjected to chromatography over silica gel C_{18} (45 g) and eluted with MeOH-Water to yield **1** (50 mg), **14** (7 mg) and **15** (11 mg). Fraction B2 (40 g) was subjected to chromatography over silica gel (400 g, 100–200 mesh) and eluted with CH_2Cl_2–MeOH to yield 12 fractions (B2-1 to B2-12) on the basis of TLC and HPLC-DAD analyses. Fraction B2-3 (1.0 g) was subjected to chromatography over silica gel (30 g, 200–300 mesh) and eluted with CH_2Cl_2-MeOH to yield 10 subfractions (B2-3-1 to B2-3-10). Subfractions were further separated on Sephadex LH-20 (MeOH) followed by semipreparative HPLC (35% aq. MeOH) to give compounds **5** (8 mg), **6** (23 mg), **7** (20 mg), **8** (12 mg), **9** (10 mg), **10** (27 mg), **11** (12 mg), **12** (120 mg) and **13** (17 mg).

Gusanlung E (**1**): yellow crystals; $[\alpha]_D^{20}$ −20 (*c* 0.03, MeOH); $UV_{\lambda max}$ (MeOH) nm 210.0, 287.0; $IR_{\nu max}$ (KBr): 3179 (OH), 3042, 2361, 1617 (C=O), 1532 cm^{-1}; CD (MeOH) Δε (nm): −9.35 (239), −1.34 (290); ^{1}H- and ^{13}C-NMR data, see Table 1; HRESIMS *m/z* 328.1581 $[M]^+$ (calcd for $C_{19}H_{22}NO_4^+$, 328.1577).

Berberine (**2**): yellow crystals; HRESIMS *m/z* 336.1205 $[M]^+$ (calcd for $C_{20}H_{18}NO_4^+$ 336.1236); ^{1}H-NMR δ: 3.27 (2H, t, *J* = 6.0 Hz, H-5), 4.11 (3H, s, 10-OCH_3), 4.21 (3H, s, 9-OCH_3), 4.94 (2H, t, *J* = 6.0 Hz, H-6), 6.11 (2H, s, -OCH_2O-), 6.97 (1H, s, H-4), 7.67 (1H, s, H-1), 8.01(1H, d, *J* = 9.0 Hz, H-12), 8.12 (1H, d, *J* = 9.0 Hz, H-11), 8.71 (1H, s, H-13), 9.77 (1H, s, H-8); ^{13}C-NMR δ: 26.4 (C-5), 55.2 (C-6), 57.1 (10-OCH_3), 62.0 (9-OCH_3), 102.1 (-OCH_2O-), 105.4 (C-1), 108.4 (C-4), 120.2 (C-13), 120.4 (C-1a), 121.4 (C-8a), 123.5 (C-12), 126.7 (C-11), 130.6 (C-4a), 132.9 (C-12a), 137.4 (C-13a), 143.6 (C-9), 145.4 (C-8), 147.6 (C-2), 149.7 (C-3), 150.4 (C-10).

Thalifendine (**3**): faint yellow powder; HRESIMS *m/z* 322.1058 $[M]^+$ (calcd for $C_{19}H_{16}NO_4^+$ 322.1074); ^{1}H-NMR δ: 3.24 (2H, t, *J* = 6.0 Hz, H-5), 4.16 (3H, s, 9-OCH_3), 4.90 (2H, t, *J* = 6.0 Hz, H-6), 6.10 (2H, s, -OCH_2O-), 6.95 (1H, s, H-4), 7.63 (1H, s, H-1), 7.88 (1H, d, *J* = 9.0 Hz, H-12), 7.78 (1H, d, *J* = 9.0 Hz, H-11), 8.64 (1H, s, H-13), 9.77 (1H, s, H-8); ^{13}C-NMR δ: 28.3 (C-5), 57.1 (C-6), 62.4 (9-OCH_3), 103.6 (-OCH_2O-), 106.4 (C-1), 109.4 (C-4), 121.7 (C-13), 122.0 (C-1a), 124.4 (C-8a), 124.7 (C-12), 132.4 (C-11), 131.6 (C-4a), 135.3 (C-12a), 139.4 (C-13a), 143.2 (C-9), 145.2 (C-8), 150.0 (C-2), 152.1 (C-3), 150.8 (C-10).

Palmatine (**4**): a faint yellow powder; HRESIMS *m/z* 352.1547 $[M]^+$ (calcd for $C_{21}H_{22}NO_4^+$ 352.1543); ^{1}H-NMR δ: 3.29 (2H, t, *J* = 6.0 Hz, H-5), 3.79 (3H, s, 2-OCH_3), 3.81 (3H, s, 3-OCH_3), 3.83 (3H, s, 9-OCH_3), 3.84 (3H, s, 10-OCH_3), 4.97 (2H, t, *J* = 6.0 Hz, H-6), 7.01 (1H, s, H-4), 7.66 (1H, s, H-1), 7.97 (1H, d, *J* = 9.0 Hz, H-12), 8.10 (1H, d, *J* = 9.0 Hz, H-11), 8.80 (1H, s, H-13), 9.79 (1H, s, H-8); ^{13}C-NMR δ: 27.3 (C-5), 56.6 (C-6), 57.2 (2-OCH_3), 57.3 (3-OCH_3), 57.3 (10-OCH_3), 63.0 (9-OCH_3), 104.7 (C-1), 110.5 (C-4), 121.5 (C-13), 121.9 (C-1a), 120.1 (C-8a), 124.4 (C-12), 126.9 (C-11), 132.6 (C-4a), 126.9 (C-12a), 138.4 (C-13a), 153.7 (C-9), 145.3 (C-8), 148.9 (C-2), 149.7 (C-3), 144.6 (C-10).

Stephabine (**5**): faint yellow powder; HRESIMS *m/z* 368.1486 $[M]^+$ (calcd for $C_{21}H_{22}NO_5^+$ 368.1498); ^{1}H-NMR δ: 3.24 (2H, t, *J* = 5.4 Hz, H-5), 3.95 (3H, s, 2-OCH_3), 3.93 (3H, s, 3-OCH_3), 3.99 (3H, s, 10-OCH_3), 4.00 (3H, s, 11-OCH_3), 4.76 (2H, t, *J* = 5.4 Hz, H-6), 6.90 (1H, s, H-4), 7.00 (1H, s, H-12) , 7.62 (1H, s, H-9), 8.84 (1H, s, H-13), 9.14 (1H, s, H-8).

8-Oxyberberine (**6**): faint yellow powder; HRESIMS *m/z* 352.1200 $[M+H]^+$ (calcd for $C_{20}H_{17}NO_5$ 352.1185); ^{1}H-NMR δ: 2.83 (2H, t, *J* = 6.6 Hz, H-5), 3.78 (3H, s, 10-OCH_3), 3.84 (3H, s, 9-OCH_3), 3.89 (2H, t, *J* = 6.6 Hz, H-6), 6.01 (2H, s, -OCH_2O-), 6.69 (1H, s, H-4), 6.95 (1H, s, H-1), 6.53 (1H, d, *J* = 9.0 Hz, H-12), 7.02 (1H, d, *J* = 9.0 Hz, H-11), 7.35 (1H, s, H-13), 8.03 (1H, s, N-H); ^{13}C-NMR δ: 29.8 (C-5), 38.8 (C-6), 56.2 (10-OCH_3), 60.9 (9-OCH_3), 102.3 (-OCH_2O-), 103.6 (C-13), 104.9 (C-1), 109.6 (C-4), 109.7 (C-11), 119.7 (C-13a), 124.6 (C-12), 126.4 (C-8a), 129.8 (C-4a), 134.0 (C-12a), 135.9 (C-1a), 148.1 (C-2), 148.8 (C-3), 149.6 (C-10), 153.0 (C-9), 160.3 (C-8).

Tetrahydropalmatine (**7**): faint yellow powder; HRESIMS *m/z* 356.1862 $[M+H]^+$ (calcd for $C_{21}H_{26}NO_4$ 356.1862); ^{1}H-NMR δ: 2.68 (2H, m, H-5), 2.84 (1H, dd, $J_{13\beta,\ 13a}$ = 13.0 Hz, $J_{13\beta,\ 13\alpha}$ = 15.0 Hz, H-13α), 3.20 (1H, dd, $J_{13\alpha,\ 13a}$ = 3.6 Hz, $J_{13\alpha,\ 13\beta}$ = 15.6 Hz, H-13β), 3.23 (2H, m, H-6), 3.53 (1H, d, *J* = 15.6 Hz, H-8α), 3.59 (1H, dd, $J_{13a,\ 13\beta}$ = 12.0 Hz, $J_{13a,\ 13\alpha}$ = 3.6 Hz, H-13a), 3.84 (6H, s, 9-OCH_3, 10-OCH_3), 3.86 (3H, s, 2-OCH_3), 3.88 (3H, s, 3-OCH_3), 4.26 (1H, d, *J* = 15.6 Hz, H-8β), 6.61 (1H, s, H-4), 6.75 (1H, s, H-1), 7.70 (1H, d, *J* = 9.0 Hz, H-11), 7.85 (1H, d, *J* = 9.0 Hz, H-12).

8-Oxotetrahydroplamatine (**8**): faint yellow powder; HRESIMS *m/z* 370.2023 $[M+H]^+$ (calcd for $C_{21}H_{24}NO_5$ 370.1654); ^{1}H-NMR δ: 2.78 (1H, dd, $J_{13\beta,\ 13a}$ = 13.0 Hz, $J_{13\beta,\ 13\alpha}$ = 15.0 Hz, H-13β), 2.80 (1H, dd, $J_{13\alpha,\ 13a}$ = 3.0 Hz, $J_{13\alpha,\ 13\beta}$ = 15.0 Hz, H-13α), 2.92 (2H, m, H-5), 3.02 (1H, m, H-6α), 3.90 (9H, s, 3 × OCH_3), 4.02 (3H, s, OCH_3), 4.70 (1H, m, H-6β), 5.05 (1H, dd, $J_{13a,\ 13\beta}$ = 9.0 Hz, $J_{13a,\ 13\alpha}$ = 2.0 Hz, H-13a), 6.67 (1H, s, H-4), 6.68 (1H, s, H-1), 6.95 (1H, d, *J* = 9.0 Hz, H-12), 7.00 (1H, d, *J* = 9.0 Hz, H-11); ^{13}C-NMR δ: 29.8 (C-5), 38.0 (C-13), 39.2 (C-6), 54.5 (C-13a), 56.2 (3 × OCH_3), 61.5 (OCH_3), 109.5 (C-1), 111.5 (C-4), 115.5 (C-11), 120.6 (C-12), 123.0 (C-8a), 127.3 (C-12a), 127.8 (C-4a), 130.7 (C-1a), 147.9 (C-3), 148.0 (C-2), 150.7 (C-10), 153.4 (C-9), 162.7 (C-8).

Gusanlung C (**9**): faint yellow powder; HRESIMS *m/z* 314.1395 $[M+H]^+$ (calcd for $C_{18}H_{20}NO_4$ 314.1392); ^{1}H-NMR δ: 2.70 (2H, t, *J* = 7.2 Hz, H-5), 3.44 (2H, t, *J* = 7.2 Hz, H-6), 3.82 (3H, s, $COOCH_3$), 6.46 (1H, d, *J* = 15.6 Hz, H-13a), 6.70 (2H, d, *J* = 8.0 Hz, H-9, H-11), 6.79 (1H, d, *J* = 8.4 Hz, H-4), 6.98 (1H, dd, *J* = 8.4, 2.0 Hz, H-3), 7.01(2H, d, *J* = 8.0 Hz, H-8a, H-12), 7.10(1H, d, *J* = 2.0 Hz, H-1), 7.41 (1H, d, *J* = 15.6 Hz, H-13), 8.00 (1H, t, *J* = 7.2 Hz, H-7); ^{13}C-NMR δ: 36.8 (C-5), 40.2 (C-6), 56.5 ($COOCH_3$), 111.6 (C-13a), 116.0 (C-8a, C-12), 116.1 (C-4), 119.7 (C-1), 122.5 (C-2), 128.0 (C-1a), 130.3 (C-9, C-11), 131.0 (C-4a), 140.7 (C-13), 148.6 (C-12a), 149.0 (C-10), 156.7 (C-2), 167.0 (C-8).

Gusanlung B (**10**): faint yellow powder; HRESIMS *m/z* 353.1252 $[M]^+$ (calcd for $C_{20}H_{19}NO_5$ 353.1263); ^{1}H-NMR δ: 2.76 (1H, dd, $J_{13\beta,\ 13a}$ = 13.0 Hz, $J_{13\beta,\ 13\alpha}$ = 15.0 Hz, H-13β), 2.68 (1H, dd, $J_{13\alpha,\ 13a}$ = 3.0 Hz, $J_{13\alpha,\ 13\beta}$ = 15.0 Hz, H-13α), 2.83 (2H, m, H-5), 2.97 (1H, m, H-6α), 3.86 (3H, s, 9-OCH_3), 4.01 (3H, s, 10-OCH_3), 4.65 (1H, dd, $J_{13a,\ 13\beta}$ = 13.0 Hz, $J_{13a,\ 13\alpha}$ = 3.0 Hz, H-13a), 4.92 (1H, m, H-6β), 5.96 (2H, s, OCH_2O), 6.65 (1H, s, H-4), 6.67 (1H, s, H-1), 6.93 (1H, d, *J* = 9.0 Hz, H-12), 7.02 (1H, d, *J* = 9.0 Hz, H-11); ^{13}C-NMR δ: 29.0 (C-5), 38.2 (C-13), 39.2 (C-6), 55.5 (C-13a), 56.2 (9-OCH_3), 61.5 (10-OCH_3), 101.5 (OCH_2O), 106.5 (C-1), 108.5 (C-4), 115.8 (C-11), 121.5 (C-12), 125.9 (C-8a), 128.7 (C-4a), 128.9 (C-12a), 131.0 (C-1a), 146.5 (C-2), 146.6 (C-3), 150.1 (C-10), 153.3 (C-9), 162.4 (C-8).

Jatrorrhizine (**11**): faint yellow powder; HRESIMS *m/z* 338.1396 $[M]^+$ (calcd for $C_{20}H_{20}NO_4^+$ 338.1392); ^{1}H-NMR δ: 3.23 (2H, t, *J* = 6.0 Hz, H-5), 4.04 (3H, s, 2-OCH_3), 4.18 (3H, s, 9-OCH_3), 4.15 (3H, s, 10-OCH_3), 4.95 (2H, t, *J* = 6.0 Hz, H-6), 7.46 (1H, s, H-4), 7.80 (1H, s, H-1), 8.08 (1H, d, *J* = 9.0 Hz, H-12), 8.02 (1H, d, *J* = 9.0 Hz, H-11), 8.81 (1H, s, H-13), 9.70 (1H, s, H-8); ^{13}C-NMR δ: 26.8 (C-5), 57.2 (C-6), 56.5 (2-OCH_3), 62.2 (9-OCH_3), 115.5 (C-1), 112.5 (C-4), 119.8 (C-13b), 121.5 (C-13), 122.9 (C-12a), 123.0 (C-12), 123.8 (C-11), 130.3 (C-4a), 135.0 (C-8a), 139.4 (C-13a), 144.6 (C-10), 145.3 (C-8), 148.9 (C-2), 149.9 (C-3), 151.7 (C-9).

8,13-Dioxo-14-hyroxycanadine (**12**): faint yellow powder; HRESIMS *m/z* 406.0900 $[M+Na]^+$ (calcd for $C_{20}H_{17}NO_7Na$ 406.0903); ^{1}H-NMR δ: 2.97–3.01 (1H, m, H-5a), 3.51–3.55 (1H, m, H-5b), 3.42 (1H,

m, H-6a), 3.87 (3H, s, 9-OCH_3), 3.89 (3H, s, 10-OCH_3), 4.15 (1H, m, H-6b), 5.96 (2H, s, -OCH_2O-), 6.67 (1H, s, H-4), 6.81 (1H, s, H-1), 7.29 (1H, d, J = 8.4 Hz, H-12), 7.53 (1H, d, J = 8.4 Hz, H-11); ^{13}C-NMR δ: 31.8 (C-5), 39.5 (C-6), 57.5 (10-OCH_3), 62.8 (9-OCH_3), 92.2 (-OCH_2O-), 103.9 (C-13a), 109.6 (C-12), 110.7 (C-11), 118.8 (C-4), 121.8 (C-1), 124.5 (C-12a), 132.6 (C-8a), 135.4 (C-1a), 138.1 (C-4a), 147.9 (C-2), 148.9 (C-3), 153.4 (C-10), 156.2 (C-9), 168.6 (C-8), 203.9 (C-13).

8,13-Dioxo-14-methoxycanadine (**13**): faint yellow powder; HRESIMS *m/z* 397.1158 $[M]^+$ (calcd for $C_{21}H_{19}NO_7$ 397.1162); ^{1}H-NMR δ: 2.78–2.80 (2H, m, H-5), 3.16 (3H, s, 14-OCH_3), 3.21 (1H, m, H-6α), 3.92 (3H, s, 9-OCH_3), 3.98 (3H, s, 10-OCH_3), 4.93 (1H, m, H-6β), 5.98 (2H, s, -OCH_2O-), 6.73 (1H, s, H-4), 6.87 (1H, s, H-1), 7.37 (1H, d, J = 9.0 Hz, H-12), 7.74 (1H, d, J = 9.0 Hz, H-11).

Corydaline (**14**): faint yellow powder; HRESIMS *m/z* 370.2011 $[M+H]^+$ (calcd for $C_{22}H_{28}NO_4$ 370.2018); ^{1}H-NMR δ: 0.95 (3H, d, J = 7.2 Hz, CH_3), 2.60 (2H, m, H-5), 3.11 (2H, m, H-6), 3.23 (1H, m, H-13), 3.52 (1H, d, J = 15.6 Hz, H-8α), 3.71 (1H, d, J = 2.4 Hz, H-13a), 3.89 (12H, m, OCH_3 × 4), 4.16 (1H, d, J =15.6 Hz, H-8β), 6.62 (1H, s, H-4), 6.67 (1H, s, H-1), 6.82 (1H, d, J = 9.0 Hz, H-11), 6.90 (1H, d, J = 9.0 Hz, H-12); ^{13}C-NMR δ: 18.2 (13-CH_3), 29.2 (C-5), 38.2 (C-13), 51.3 (C-6), 54.0 (C-8), 55.5 (-OCH_3), 55.6 (-OCH_3), 56.0 (-OCH_3), 60.0 (-OCH_3), 63.0 (C-13a), 108.7 (C-1), 110.8 (C-4), 111.1 (C-11), 123.8 (C-12), 128.4 (C-4a, C-8a, C-1a), 134.8 (C-12a), 146.0 (C-10), 147.1 (C-2), 147.5 (C-3), 145.0 (C-9).

Tetrahydrothalifendine (**15**): faint yellow powder; HRESIMS *m/z* 326.1395 $[M+H]^+$ (calcd for $C_{19}H_{20}NO_4$ 326.1392); ^{1}H-NMR δ: 2.68 (2H, m, H-5), 2.84 (1H, dd, $J_{13\beta,\ 13a}$ = 12.6 Hz, $J_{13\beta,\ 13\alpha}$ = 15.6 Hz, H-13α), 3.13 (1H, dd, $J_{13\alpha,\ 13a}$ = 3.6 Hz, $J_{13\alpha,\ 13\beta}$ = 15.6 Hz, H-13β), 3.32 (2H, m, H-6), 3.53 (1H, d, J = 15.6 Hz, H-8α), 3.57(1H, dd, $J_{13a,\ 13\beta}$ = 12.6 Hz, $J_{13a,\ 13\alpha}$ = 3.6 Hz, H-13a), 3.91(3H, s, OCH_3), 4.15(1H, d, J = 15.6 Hz, H-8β), 5.96 (2H, s, -OCH_2O-), 6.63 (1H, s, H-4), 6.67 (1H, s, H-1), 6.62 (1H, d, J = 9.0 Hz, H-11), 6.90 (1H, d, J = 9.0 Hz, H-12); ^{13}C-NMR δ: 29.2 (C-5), 36.2 (C-13), 51.5 (C-6), 53.0 (C-8), 59.0 (C-13a), 55.1 (-OCH_3), 101.3 (-OCH_2O-),106.7 (C-1), 110.8 (C-4), 111.3 (C-11), 121.7 (C-12), 125.9 (C-8a), 127.1 (C-12a), 127.8 (C-4a), 131.0 (C-1a), 143.0 (C-10), 144.1 (C-2), 144.0 (C-3), 145.0 (C-9).

3.4. Cytotoxicity Testing

The cytotoxicity of the compounds was determined using the colorimetric methylthiazoletetrazolium (MTT) assay with taxol as the positive control (IC_{50} value 0.15 μM). The human stomach cancer cell line SGC 7901 in logarithmic phase were seeded in 96 well flat bottom microtitre plates at a density of 1×10^4 cells per well. cells were washed and maintained with different concentrations of drug, 10 μL MTT was added to the culture medium to a final concentration of 0.5 mg/mL and incubated at 37 °C for 4 h. Formazan crystals dissolved in 100 μL DMSO was added and 10 min later the absorbance of the solution was measured at a wavelength of 570 nm. All assays were carried out in triplicate.

4. Conclusions

From the chemical investigation of stems of *A. gusanlung*, fifteen protoberberine alkaloids including a new one, named gusanlung E (**1**), were isolated and identified. Gusanlung E (**1**) showed weak

cytotoxicity against cancer cell line SGC 7901. These analogues should be studied in more advanced models to establish *in vivo* efficacy.

Supplementary Materials

Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/19/9/13332/s1.

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Author Contributions

Y.L.L. and Z.Z.M. designed the research; Y.L.L., L.R.T., A.Y.B., L.W. and D.Z.S. performed the experimental work; Y.L.L. wrote the manuscript. All authors discussed, edited and approved the final version.

Conflicts of Interest

The authors declare no conflict of interest.

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Sample Availability: Samples of the compounds are not available from the authors because bioactivity tests of those compounds are going on.