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Article

Expanding the Chemical Diversity of *Stemona parviflora*: Isolation and Characterization of New Parvistemoline-Type Alkaloids

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Abstract: A comprehensive phytochemical investigation of the whole plant of *Stemona parviflora* led to the isolation of 13 alkaloidal constituents, including five new alkaloids: 13 α -hydroxyparvistemonine (1), 12 α -methoxyparvistemonine (2), parvistemonine-N-oxide (3), parvistemoninine (4), and parvistemofoline (5). The structures of these compounds were elucidated through extensive analyses of 1D and 2D NMR spectra, DFT NMR calculation, and comparisons with data in literature. Notably, compounds 1–4 represent new examples of the rare parvistemoline-type alkaloids, with compound 4 showcasing a unique rearranged skeleton. Additionally, parvistemofoline (5) was identified as a distinct alkaloid skeleton characterized by a n-butyl side chain. These findings significantly expand our understanding of the chemical diversity of parvistemoline-type alkaloids, and provide clues for elucidating the biosynthetic pathways of these structurally unique parvistemoline alkaloids.

Keywords: *Stemona parviflora*; alkaloid; parvistemoline-type; 13 α -hydroxyparvistemonine; 12 α -methoxyparvistemonine; parvistemonine-N-oxide; parvistemofoline; parvistemoninine

1. Introduction

Stemonaceae is a small family of monocotyledonous flowering plants within the order Pandanales, consisting of four genera: *Croomia*, *Stemona*, *Stichoneuron*, and *Pentastemona*, with about 37 known species [1,2]. These plants are native to Southeast Asia, Northern Australia, China, Japan, and Northern America [1,3,4]. *Stemona* is the largest genus with approximately 25–32 species, known for its unique alkaloids, which typically feature either a pyrrolo- or a pyrido[1,2- α]azepine core. To date, over 250 alkaloids of various structural types have been isolated from different *Stemona* species, with previous research demonstrating that these alkaloids exhibited various biological activities such as antitussive, insecticidal, antifeedant, nematocidal, and repellent properties [5,6].

Stemona parviflora C. H. Wright is a species endemic mostly in Hainan and Guangdong Province, China [7]. It has a long-standing history of use in Chinese folk medicine, commonly referred to as "Baibu," where it is employed to treat respiratory disorders, including pulmonary tuberculosis and bronchitis, as well as for its insecticidal properties [7]. Despite its traditional uses, there have been relatively few chemical investigations into this species in recent years [8–14]. These studies revealed the existence of minor phenylethyl benzoquinones and phenanthrenes in addition to the predominant alkaloids. A unique structural type of alkaloid, belonging to the parvistemoline group [15,16], has been identified in this species. This group, with only 13 derivatives reported so far, is characterized by the presence of a substituent at the C-9 position of the pyrrolo[1,2- α]azepine nucleus

and the absence of a fused B-C ring system, which distinguishes it from other groups of *Stemona* alkaloids [6]. This limited exploration underscores the need for further research into this species.

This investigation aims to explore and identify new alkaloidal constituents from *S. parviflora*, thereby providing valuable insights into its phytochemical profile and potential therapeutic applications. A systematic chemical investigation was conducted, resulting in the isolation of a total of 13 alkaloids, including four novel parvistemoline-type compounds (**1–4**, Figure 1), one new stemofoline-type derivative (**5**, Figure 1), and eight known compounds. The structures of these compounds were elucidated through extensive analysis of spectroscopic data, including MS, IR, 1D- and 2D-NMR spectroscopic data, density functional theory (DFT) NMR calculations, as well as comparisons with existing literature.

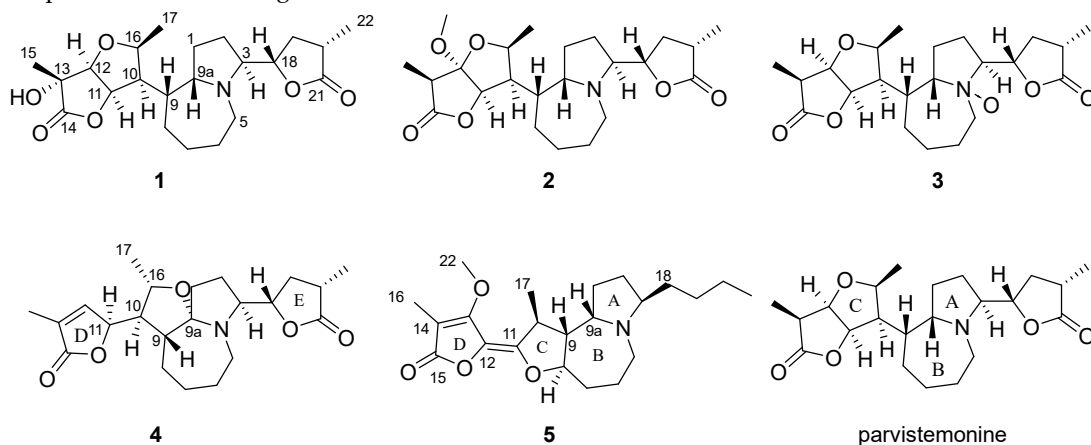


Figure 1. Structures of compounds **1–5** and parvistemonine.

2. Materials and Methods

2.1. General Experimental Procedures

Optical rotations were measured with a Perkin-Elmer 241MC polarimeter or a Perkin-Elmer 341 polarimeter. IR spectra were recorded using a Perkin-Elmer 577 Spectrometer. ESIMS were measured by using a Finnigan LCQ-DECA mass spectrometer, and HRESIMS were obtained on a Waters Q-TOF Micro MS spectrometer. EIMS and HREIMS were recorded on a Finnigan MAT-95 mass spectrometer. ^1H , ^{13}C , and 2D NMR spectra were recorded on a Bruker AM-300, Varian INOVA 400 or Bruker Avance III 500 NMR spectrometer with solvent resonances (CDCl_3 , δ_{H} 7.26; δ_{C} 77.16) as internal standards. Chemical shifts were reported in ppm (δ), with coupling constants (J) in hertz. Column chromatographic separations were carried out using silica gel (Qingdao Haiyang Chemical Group Corporation, China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) as packing materials. Precoated silica gel 60 F₂₅₄ aluminum sheets (Merck Millipore, Germany) were used for analytical TLC. Visualization of TLC spots was performed by Dragendorff's reagent and Iodine. Analytical HPLC was performed on a Waters 2695 separations module coupled with a Waters 998 DAD UV detector, a Waters Acquity® ELSD and a Waters 3100 SQD MS. The HPLC-MS analysis were performed on a Waters Sunfire® RP C₁₈, 3.5 μm , 4.6 \times 100 mm column using a gradient solvent system composed of H₂O and CH₃CN (5% to 95%) with 0.1% formic acid, at a flow rate of 1.0 mL/min.

2.2. Plant Material

The whole plants of *S. parviflora* were collected in Hainan province, China, in 2013 and identified by Qiong-Xin Zhong from Hainan Normal University. A voucher specimen (No. 20130401) has been deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

2.3. Extraction and Isolation

Air-dried roots of *S. parviflora* (10.0 kg) were grounded into powder and extracted with 95% EtOH (25 L) for three times, three days each. After evaporation of the collected filtrate, the crude extract was acidified with dilute HCl (4%) to pH 1-2 and partitioned between ethyl ether and water. The aqueous part was basified with aqueous NH_3 to pH 9-10 and extracted repeatedly with CH_2Cl_2 to afford 70 g of crude alkaloid. The crude alkaloid was subjected to column chromatography over silica gel (200-300 mesh) and eluted with petroleum ether–acetone from 4:1 to 1:4, and then acetone to yield four major fractions (XB1-XB4). Fraction XB1 was subjected to repeated column chromatography over silica gel, and then Sephadex LH-20 to afford isomaistemonine (151 mg), maistemonine (49 mg), croomine (553 mg). Similarly, fraction XB2 gave 12 α -methoxyparvistemonine (**2**, 515 mg), parvistemonifoline (**5**, 64 mg), parvineostemonine (143 mg), and parvistemonine (4.9 mg), and fraction XB3 afforded 13 α -hydroxyparvistemonine (**1**, 832 mg), parvistemonine-N-oxide (**3**, 2 mg), parvistemoninine (**4**, 64 mg), protostemonine (533 mg), and protostemoamide (5 mg). Stemofoline (680 mg) was afforded from fraction XB4.

2.3.1.13 α -. Hydroxyparvistemonine (1)

Light yellow amorphous powder; $[\alpha]_D^{20} + 4$ (c 0.1, MeOH); IR (KBr) ν_{max} 3432, 2935, 1770, 1664, 1456, 1380, 1195, 1088, 1016, 939 cm^{-1} ; ^1H and ^{13}C NMR data: see Tables 1 and 2; EIMS m/z : 407 $[\text{M}]^+$, 308 (100) $[\text{M} - 99]^+$; HREIMS m/z : 407.2305 $[\text{M}]^+$ (calcd for $\text{C}_{22}\text{H}_{33}\text{NO}_6$, 407.2308).

2.3.2.12 α -. Methoxyparvistemonine (2)

Yellow amorphous powder; $[\alpha]_D^{20} + 81$ (c 0.1, Acetone); IR (KBr) ν_{max} 2935, 1787, 1776, 1456, 1381, 1190, 1041, 991, 754 cm^{-1} ; ^1H and ^{13}C NMR data: see Tables 1 and 2; EIMS m/z : 421 $[\text{M}]^+$, 322 (100) $[\text{M} - 99]^+$; HRESIMS m/z : 422.2544 (calcd for $\text{C}_{23}\text{H}_{36}\text{NO}_6$, 422.2543).

2.3.3. Parvistemonine-N-Oxide (3)

Yellow amorphous powder; $[\alpha]_D^{20} + 9$ (c 0.1, MeOH); IR (KBr) ν_{max} : 3433, 2937, 1772, 1457, 1167, 997 cm^{-1} ; HRESIMS m/z : 408.2385 (calcd for $\text{C}_{22}\text{H}_{34}\text{NO}_6$, 408.2386). ^1H -NMR (300 MHz, CDCl_3): δ_{H} 4.91 (dd, 1H, $J = 3.8, 3.9$ Hz; H-11), 4.61 (m, 1H; H-12), 4.32 (m, 1H; H-16), 4.05 (m, 1H; H-18), 3.85 (m, 1H; H-5 β), 3.70 (m, 3H; H-3, H-5 α and H-9a), 2.70 (m, 3H; H-13, H-19, H-20), 2.50 (m, 1H; H-9), 2.20-1.50 (m, 10H; H-1, H-2, H-6, H-7, and H-8), 1.30 (m, 9H; Me-15, Me-17, Me-22); ^{13}C -NMR (100 MHz, CDCl_3): δ_{C} 24.8 (t, C-1); 24.1 (t, C-2), 76.9 (d, C-3), 69.0 (t, C-5), 23.5 (t, C-6), 26.7 (t, C-7), 28.1 (t, C-8), 34.9 (d, C-9), 73.9 (d, C-9a), 49.7 (d, C-10), 89.6 (d, C-11), 79.8 (d, C-12), 41.2 (d, C-13), 178.2 (s, C-14), 18.6 (C-15), 77.1 (d, C-16), 9.0 (q, C-17), 83.0 (d, C-18), 34.8 (t, C-19), 34.2 (d, C-20), 178.5 (s, C-21), 14.9 (q, C-22). The signals were assigned according to the proton and carbon assignments of the known compound parvistemonine.

2.3.4. Parvistemoninine (4)

Light yellow amorphous powder; $[\alpha]_D^{20} - 4$ (c 0.2, Acetone); IR (KBr) ν_{max} 2929, 1759, 1668, 1456, 1334, 1163, 754 cm^{-1} ; ^1H and ^{13}C NMR data: see Tables 1 and 2; ESI-MS m/z : 390.4 $[\text{M} + \text{H}]^+$, EIMS m/z 389 $[\text{M}]^+$, 290 (100) $[\text{M} - 99]^+$; HRESIMS m/z : 390.2281 (calcd for $\text{C}_{22}\text{H}_{32}\text{NO}_5$, 390.2280).

2.3.5. Parvistemonifoline (5)

Yellow amorphous powder; $[\alpha]_D^{20} - 79$ (c 0.1, Acetone); IR (KBr) ν_{max} 2931, 1739, 1680, 1616, 1460, 1398, 1215, 1155, 1063, 1016, 754 cm^{-1} ; ^1H and ^{13}C NMR data: see Table 3; EIMS m/z 375 $[\text{M}]^+$, 318, 265, 208 (100); HRESIMS m/z : 376.2491 (calcd for $\text{C}_{22}\text{H}_{34}\text{NO}_4$, 376.2488).

3. Results

Compound **1** was obtained as light-yellow amorphous powder, positive to the Dragendorff's reagent. Its molecular formula was established by HREIMS (m/z 407.2305 $[M]^+$, calcd 407.2308) as $C_{22}H_{33}NO_6$ with seven degrees of unsaturation. The 1H NMR spectrum (Table 1) displayed signals of a methylene (δ_H 2.87, 3.40) characteristic for the CH_2 -5 of the pyrrolo[1,2- α]azepine nucleus in *Stemona* alkaloids [17], two doublet methyls (δ_H 1.15, Me-17; 1.25, Me-22), and one singlet methyl (δ_H 1.50, Me-15). In the ^{13}C NMR spectrum two quaternary carbons at δ_C 179.8 and 177.6 suggested the presence of two γ -lactone rings. The base peak at 308 $[M - 99]^+$ in EIMS further confirmed the presence of an α -methyl- γ -lactone moiety attached to C-3 [18,19]. All these data were indicative of a parvistemonine-type skeleton of compound **1**. A further NMR data comparison of compound **1** and the known parvistemonine [12,20] revealed big similarities of rings A, B, C and E between these two compounds except that an oxygenated quaternary carbon (δ_C 76.1, C-13) and a singlet methyl instead of a doublet methyl in parvistemonine were observed in compound **1**. Considering the molecular formula of **1** contains one more oxygen atom than that of parvistemonine, compound **1** was deduced to be a 13-hydroxyl derivative of parvistemonine. Such elucidation was further confirmed by 1H - 1H COSY, HSQC and HMBC spectra (Figures S3-S5). The ROESY correlation between Me-15 and Me-17 suggested an α -orientation of OH-13 (Figure S6). Therefore, the structure of compound **1** was fully determined as 13 α -hydroxyparvistemonine.

Compound **2**, yellow amorphous powder, was positive to the Dragendorff's reagent. The pseudo molecular ion at m/z 422.2544 ($[M + H]^+$) in the HRESIMS suggested a molecular formula of $C_{23}H_{35}NO_6$, corresponding to seven degrees of unsaturation. Its EIMS base peak at m/z 322 ($[M - 99]^+$) indicated also the existence of an α -methyl- γ -lactone moiety. Further analysis of its NMR data (Tables 1 and 2) revealed that compound **2** was also a parvistemonine-type alkaloid: both 1H and ^{13}C NMR data of **2** were quite similar to those of the known parvistemonine and compound **1**, except for the presence of an extra methoxy group (δ_H 3.35, MeO-23) in **2**, which caused some chemical shift differences at C-12 and C-13. In the ^{13}C NMR and DEPT spectra (Figures S9 and S10), a quaternary carbon signal resonating at δ_C 110.3 replaced the methine at C-12 in parvistemonine and compound **1**, suggesting the methoxy group might be located at C-12. HMBC correlations from MeO-23, Me-15, and H-16 to C-12 further supported the assumption. The relative configuration of **2** was determined by the NOESY experiment, and the cross-peaks of H-11/MeO-23 and H-11/H-13 indicated an α -orientation of the methoxy group. Therefore, the structure of compound **2** was established, and named 12 α -methoxyparvistemonine.

Compound **3** was also obtained as yellow amorphous powder, and positive to the Dragendorff's reagent. The molecular formula was established as $C_{22}H_{33}NO_6$ (m/z 408.2385, $[M + H]^+$) by HRESIMS, one more oxygen atom than that of parvistemonine. The 1H NMR data (Table 1) displayed signals of three doublet methyls (δ_H 1.28-1.30, 9H, overlapped), indicative of also a parvistemonine skeleton. The ^{13}C NMR data (Table 2) highly resembled those of parvistemonine, suggesting that the additional oxygen atom was not substituted at any carbon of the skeleton but the nitrogen atom. Accordingly, compound **3** was finally proposed as *N*-oxide derivative of parvistemonine, and named parvistemonine-*N*-oxide.

Table 1. 1H NMR data (300 MHz) for compounds **1**, **2** and **4** in $CDCl_3$.

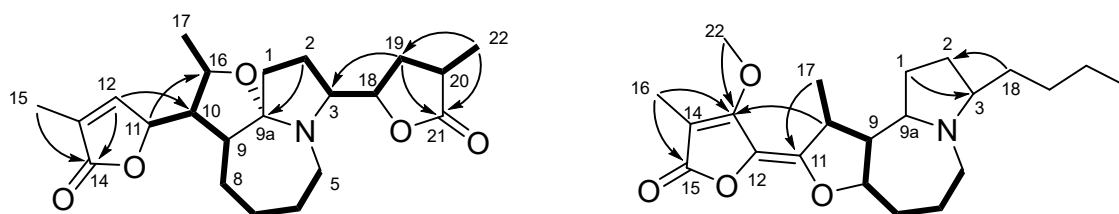
No.	1	2	4
1	1.42, m	1.40, m	1.67, m
	1.64, m	1.64, m	1.90, m
2	1.59, m	1.59, m	1.42, m
	1.75, m	1.75, m	2.05, m
3	3.45, m	3.40, m	3.16, m
5	2.87, dd (15.7, 11.9)	2.85, d (11.7)	2.99, dd (14.4, 11.4)
	3.40, m	3.30, dd (11.7, 7.9)	2.74, d (14.4)

6	1.44, m	1.42, m	1.59, m
	1.72, m	1.70, m	1.65, m
7	1.42, m;	1.38, m	1.42, m;
	1.82, m	1.77, m	1.61, m
8	1.46, m;	1.45, m	1.60, m
	1.92, m	1.91, m	1.71, m
9	2.18, m	2.15, m	2.38, m
9a	3.45, m	3.43, m	
10	2.15, m	2.30, m	2.01, m
11	5.14, d (3.8)	4.61, d (4.3)	4.84, dd (3.4, 1.7)
12	4.25, d (3.8)		7.02, d (1.7)
13		2.89, m	
15	1.50, s, 3H	1.34, d (7.2)	1.92, s
16	4.30, m	4.48, m	3.55, m
17	1.15, d (6.7), 3H	1.20, d (6.6)	1.28, d (5.9)
18	4.20, m	4.17, ddd (10.8, 7.1, 5.3)	4.22, m
19	1.55, m	1.55, m	2.36, m
	2.37, m	2.35, m	1.49, m
20	2.61, m	2.59, m	2.62, m
22	1.25, d (7.1), 3H	1.25, d (7.0), 3H	1.24, d (7.0), 3H
23-OMe		3.35, s, 3H	

The molecular formula of compound **4** was established as $C_{22}H_{31}NO_5$ according to the pseudo molecular ion at m/z 390.2281 ($[M + H]^+$) in the HRESIMS and the ^{13}C NMR data, corresponding to eight degrees of unsaturation. Its 1H NMR data (Table 3) showed the characteristic signals of a methylene (δ_H 2.99, 2.74), indicative of a pyrrolo[1,2- α]azepine nucleus (rings A and B). The ^{13}C NMR and DEPT 135 spectra (Table 3, Figures S21-S22) showed resonances ascribed to three methyl, seven methylene, eight methine (one olefinic at δ_C 147.3, and three oxygenated at δ_C 71.3, 80.9 and 82.2), and four quaternary carbons (one olefinic at δ_C 130.6, one oxygenated at δ_C 105.1, and two carbonyl at δ_C 173.8 and 179.9). In the EIMS spectrum, the base peak at m/z 290 $[M - 99]^+$ suggested the presence of an α -methyl- γ -lactone moiety. IR absorptions at 1668 cm^{-1} , together with the carbonyl resonating at δ_C 173.8, indicated the presence of another α,β -unsaturated α -methyl- γ -lactone ring. The 1H - 1H COSY spectrum (Figure S23) revealed two spin systems of $-C(1)H_2-C(2)H_2-C(3)H-C(18)H-C(19)H_2-C(20)H-C(22)H_3$ and $-C(5)H_2-C(6)H_2-C(7)H_2-C(8)H_2-C(9)H-C(10)H(CH)-C(16)H-C(17)H_3$ (Figure 2). The former moiety, in combination with the HMBC correlations from Me-22 and H-19 to the carbonyl carbon (C-21) revealed the α -methyl- γ -lactone (ring E) attached to C-3. HMBC correlations from H-12 and Me-15 to another carbonyl carbon C-14, from H-12 to C-10, and from H-11 to C-16 suggested the α,β -unsaturated lactone ring (ring D) connected to C-10 (Figure 2). Since seven degrees of unsaturation were occupied by the pyrrolo[1,2- α]azepine nucleus and the identified rings D and E, the remaining one indicated the presence of another ring. Given that C-12 was not oxygenated, together with the obvious down-fielded chemical shifts of C-9a and C-9, the last ring was eventually constructed by forming an oxygen bridge between C-9a and C-16. Therefore, the planar structure of **4** was established.

Table 2. ^{13}C NMR data (125 MHz) for compounds **1**, **2** and **4** in CDCl_3 .

No.	1	2	4
1	27.0 t	27.1 t	38.6 t
2	26.8 t	26.9 t	23.7 t
3	63.7 d	63.7 d	67.0 d
5	46.5 t	46.6 t	46.0 t
6	24.5 t	24.8 t	23.1 t
7	28.6 t	28.7 t	31.2 t
8	26.7 t	26.9 t	27.0 t
9	39.1 d	39.0 d	46.5 d
9a	62.8 d	62.9 d	105.1 s
10	49.4 d	47.4 d	50.3 d
11	84.0 d	86.4 d	80.9 d
12	84.4 d	110.3 s	147.3 d
13	76.1 s	44.7 d	130.6 s
14	177.6 s	176.3 s	173.8 s
15	18.6 q	10.0 q	10.8 q
16	77.9 d	79.4 d	71.3 d
17	19.3 q	19.4 q	20.2 q
18	83.3 d	83.4 d	82.2 d
19	34.2 t	34.2 t	33.7 t
20	34.9 d	35.0 d	35.4 d
21	179.8 s	179.7 s	179.9 s
22	14.9 q	15.0 q	15.2 q
23-OMe		51.2 q	

**Figure 2.** Important ^1H - ^1H COSY (—) and HMBC (H \rightarrow C) correlations of compounds **4** (left) and **5** (right).

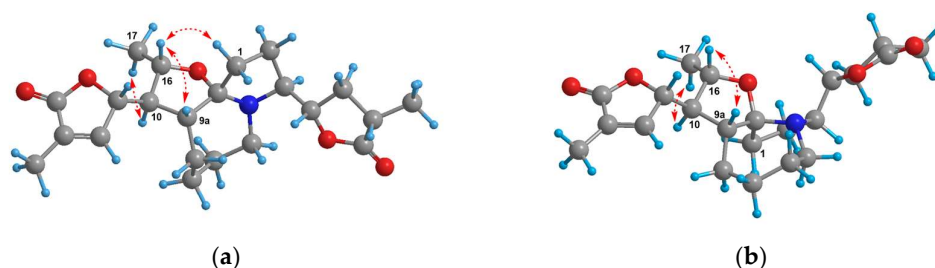


Figure 3. 3D structure models of two presumed configurations of compound 4, and key NOESY correlations (H \leftrightarrow H). (a) the C-O bond at C-9a was α -orientated; (b) the C-O bond at C-9a was β -orientated (generated by ChemBio3D software).

The relative configuration was inferred from the NOESY spectrum and biogenetic consideration. The cross-peaks of H-16/H-9 and Me-17/H-10 suggested that the configurations of C-9, C-10, and C-16 could keep untouched. Assuming the configuration at C-12 remained unchanged, there would be two possible configurations for C-9a as showed in Figure 3. Given the observable correlation between H-16 and H-1, which is only possible when the C-O bond at C-9a was in the α -orientation, we eventually proposed an *S*-configuration of C-9a.

The configuration of C-11 was assumed to also unchanged. However, due to the free rotation caused by the single bond between C-10 and C-11, the relative configuration of C-11 could not be confirmed unambiguously by the NOESY experiment. Therefore, DFT NMR calculation was performed with the Gaussian 16 program [21] on two possible conformations, 11*S*-4 and 11*R*-4 (Figure S29). Conformational search was performed using the Conflex 8.0 software within an energy window of 5.0 kcal/mol [22], and the conformers with the Boltzmann population above 1.0% were selected for re-optimization at the B3LYP/6-31G(d) level in vacuo. The ^1H and ^{13}C NMR magnetic shielding tensors of each non-redundant conformer were calculated at the level of mPW1PW91/6-311G(d,p) with the PCM solvent mode for chloroform. The final ^1H and ^{13}C NMR data for each conformation were obtained after Boltzmann weighted average. The DP4+ statistical analysis was then used for the experimental and calculated data, and the results specified the 11*S*-4 (all data, 100%) as the most probable conformation of 2 (Figure S29) [23].

Subsequently, compound 4 was established as a parvistemonine-type derivative, and named parvistemoninine. This structure features a rearranged furan ring, presumably formed through the cleavage of the ether bond of C-12 in parvistemonine, followed by the reformation of a new bond of C-16-O-C-9a. This accounts for the unchanged configurations at C-9, C-10, C-11, and C-16. This type of ring formation is a novel discovery in the *Stemona* alkaloids.

Compound 5, positive to the Dragendorff's reagent, was obtained as a yellow amorphous powder. The HRESIMS data suggested a molecular formula of $\text{C}_{22}\text{H}_{33}\text{NO}_4$ (m/z 376.2491 [$\text{M} + \text{H}$] $^+$), with an unsaturation equivalence of seven. Considering the fragment peak at m/z 318 ($[\text{M} - 57]^+$) in the EIMS (Figure S37), and a triplet methyl signal (δ_{H} 0.88, t, $J = 7.0$ Hz) in the ^1H NMR spectrum (Figure S30), an *n*-butyl side chain was proposed. The ^1H NMR data (Table 3) showed signals of one methoxy (δ_{H} 4.11) and three methyls, as well as characteristic signals of the pyrrolo[1,2-*a*]azepine nucleus presenting in *Stemona* alkaloids. The ^{13}C NMR and DEPT 135 spectra (Figures S31-S32) gave 22 resonances ascribed to four methyls (one methoxy, δ_{C} 58.9), eight methylenes, five methines (one oxygenated, δ_{C} 83.8), and five quaternary carbons (four olefinic, δ_{C} 97.1, 124.6, 149.1, 163.3; one carboxyl, δ_{C} 170.2). The ^1H - ^1H COSY spectrum (Figure S33) revealed a spin system of $-\text{C}(5)\text{H}_2-\text{C}(6)\text{H}_2-\text{C}(7)\text{H}_2-\text{C}(8)\text{H}(\text{O})-\text{C}(9)\text{H}-\text{C}(10)\text{H}-\text{C}(17)\text{H}_3$, which was in accordance with a protostemonine-type skeleton [19]. HMBC correlations from H₃-17 to C-11, H-10 to C-12, H₃-16 to C-13 and C-15, and OMe-22 to C-13 exhibited an α,β -unsaturated α -methyl- γ -lactone, connecting to ring C through the C11-C12 double bond, while the correlations from H-1 to C-3 and H-18 to C-2 confirmed the *n*-butyl was connecting to C-3. All these data evidenced that the structure of compound

5 closely resembled that of protostemonine with the exception of an *n*-butyl group being present instead of an α -methyl- γ -lactone group at C-3. Consequently, the structure of compound **5** was fully elucidated. Considering the *n*-butyl side chain was distinct in stemofoline-type alkaloids [24], compound **5** was named as parvistemofoline.

Accompanying with the new compounds, eight known alkaloids were identified as parvistemonine [12], parvineostemonine [9], protostemonine [19], croomine [2], protostemoamide [25], stemofoline [24], maistemonine [26], and isomaistemonine [27], by comparison with data in literature.

Table 3. ¹H (400 MHz) and ¹³C NMR (100 MHz) data for compounds **5** in CDCl₃.

No.	δ_H	δ_C
1	1.50, m	27.3 t
	1.93, m	
2	1.48, m	30.9 t
	2.01, m	
3	3.03, m	61.1 d
5	3.21, dd (11.7, 4.1)	44.8 t
	2.98, dd (11.7, 7.9)	
6	1.52, m	18.9 t
	1.68, m	
7	1.51, m	33.7 t
	2.34, m	
8	4.13, m	83.8 d
9	2.22, m	56.1 d
9a	3.83, m	58.3 d
10	2.92, m	39.4 d
11		149.1 s
12		124.6 s
13		163.3 s
14		97.1 s
15		170.2 s
16	2.04, s, 3H	9.2 q
17	1.33, d, 3H	20.7 q
18	1.22, m	32.4 t
	1.64, m	
19	1.25, m, 2H	28.4 t
20	1.30, m, 2H	23.0 t
21	0.88, t (7.0), 3H	14.1 q
22-OMe	4.11, s, 3H	58.9 q

4. Discussion

Stemona alkaloids are a small family of unique structures having a pyrrolo[1,2-*a*]azepine or a pyrido[1,2- *a*]azepine nucleus. Based on their structural features, the classification of these alkaloids was presented firstly by Götz and Strunz in 1975 [28]. Then, Pilli et al. proposed in 2000 and 2010 to

classify *Stemona* alkaloids into six or eight structural groups, respectively [15,16]. In this paper, we have followed the aforementioned classification method, categorizing the obtained compounds **1-4** as parvistemonine-type. This classification method effectively reflects the structural characteristics of the compounds but lacks a description of the biosynthetic relationships between different structural types.

In 2006, Greger suggested a new classification based on biosynthetic considerations, and classified the *Stemona* alkaloids into three skeletal types – the stichoneurine-type (tuberostemonine-type), protostemonine-type, and croomine-type alkaloids according to different carbon chains attached to C-9 of the pyrroloazepine nucleus [5,29]. In his classification, parvistemonine-type alkaloids were eventually classified as stemoninine-type derivatives of the stichoneurine skeleton [5]. The structure of parvistemonine is hypothesized to originate from the formation of an ether linkage between C-12 and C-16 of a stichoneurine-type compound. The rearranged structure of compound **4**, which constructed an ether linkage between C-16 and C-9a, supported that the stichoneurine-type could be the precursor of the parvistemonine alkaloids.

5. Conclusions

In this study, we successfully isolated five new alkaloids from the roots of *S. parviflora*. Among these, four compounds (**1-4**) are derivatives of the parvistemonine-type alkaloids, a relatively rare class of structures reported in previous phytochemical investigations of *Stemona* species. Notably, compound **4** represents the first identified ring-C rearranged skeleton within this group of compounds. In addition, compound **5** possesses a unique structural skeleton, representing a hybrid of protostemonine-type and stemofoline-type. Our findings expand the chemical diversity of *S. parviflora*, and provide valuable clues for the study of the biosynthetic pathways of *Stemona* alkaloids.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figures S1- S37: ¹H and ¹³C NMR of compounds **1-5**, ¹H-¹H COSY, HSQC, HMBC, and NOESY spectra of compounds **1, 2, 4, 5**, as well as the DFT NMR calculation results of compound **4**.

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