Alkaloids from *Stemona mairei*

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Abstract

Three new alkaloids, maireistemoninol (**1**), neotuberostemonone (**2**) and epoxytuberostemonone (**3**), together with six known alkaloids, neotuberostemoninol (**4**), neotuberostemonine (**5**), bisdehydroneotuberostemonine (**6**), bisdehydrotuberostemonine (**7**), 2-oxostenine (**8**), and stemotinine (**9**), were isolated from the roots of *Stemona mairei* (levl.) Krause. The structures were elucidated by a combination of 1D, 2D-NMR, and mass spectral techniques.

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Letter

Plants of the genus *Stemona* (Stemonaceae) have been used in China as insecticides and a cough remedy [1]. About eighty *Stemona* alkaloids, as unique secondary metabolites in the family Stemonaceae, have been isolated [2]. Some of them reportedly have insecticidal, antitussive, and anti-inflammatory activities [3], [4], [5]. However, up to now, few of the alkaloids were reported in *Stemona mairei* [6], which is locally distributed in Shichuan and the North of the Yunnan province [7], P. R. China. In our investigation, three new alkaloids, named maireistemoninol (**1**), neotuberostemonone (**2**) and epoxytuberostemonone (**3**), along with six known alkaloids, neotuberostemoninol (**4**) [8], neotuberostemonine (**5**) [9]. bisdehydroneotuberostemonine (**6**) [9], bisdehydrotuberostemonine (**7**) [9], [10], 2-oxostenine (**8**) [11], [12], and stemotinine (**9**) [13], were isolated from the roots of *S. mairei* (Fig. **1**). In this paper, we describe the isolation and structural elucidation of these alkaloids on the basis of spectroscopic techniques (for the spectral data of the known alkaloids, see the Supporting Information).

Maireistemoninol (**1**) was obtained as colorless needle crystals. Assignment of the molecular formula as $C_{22}H_{31}NO_6$ was based on the HR-ESI-MS data (*m/z* = 428.2045 [M + Na]$^+$, calcd.: 428.2049). Its IR (KBr) spectrum showed absorption bands for hydroxy (3477 cm^{-1}) and carbonyl groups (1762, 1760, and 1669 cm^{-1}). The ^{1}H-NMR spectrum indicated the presence of a primary methyl group at δ_H = 1.02 (3H, t, *J* = 7.5 Hz), two secondary methyl groups at δ_H = 1.15 (3H, d, *J* = 7.5 Hz) and 1.18 (3H, d, *J* = 7.5 Hz), and four low-field protons attached to carbon atoms bearing an oxygen or nitrogen at δ_H = 4.70 (1H, t, *J* = 3.7 Hz), 4.58 (1H, m), 4.53 (1H, m) and 4.06 (1H, brs). The ^{13}C-MR and DEPT spectra of **1** showed 22 carbon atoms: three lactonic carbonyl atoms [δ_C = 186.5 (s), 179.1(s) and 178.5(s)], nine methine carbons [δ_C = 80.9 (d), 78.4 (d), 77.0 (d), 74.2 (d), 50.3 (d), 44.7 (d), 43.9 (d), 42.5 (d), and 35.9 (d)], six methylene groups [δ_C = 33.6 (t), 32.0 (t), 31.8 (t), 30.1 (t), 20.2 (t), and 18.4(t)], three methyl groups [δ_C = 15.5 (q), 12.6 (q), and 11.0 (q)], and a quaternary carbon [δ_C = 51.4, (s)]. The ^{13}C-NMR and DEPT patterns of **1** were identical to those of the known compound tuberostemoninol, having the same molecular formula. Compared to the ^{13}C-NMR spectrum of tuberostemoninol [14], signals for one of the methylenes and for the quaternary carbons [δ_C = 81.7 (s)] disappeared in **1**; instead, additional two methines were present in **1** suggesting that the position of the hydroxy group in **1** was different from that in tuberostemoninol.

In the HMBC specrum of **1,** the signal at δ_H = 4.06 (1H, brs) corresponding to δ_C = 77.0 (d) in the HSQC spectrum correlated with δ_C = 31.8 (C-7, t), 51.4 (C-9, s), 186.5 (C-9a, s), and 44.7 (C-10, d) (Table **1**) thereby placing the hydroxy group at C-8, which was confirmed by cross-peaks between H-8 (δ_H = 4.06, brs) and H-7 [δ_H = 1.92 (m), 1.68 (m)], H-6 (δ_H = 1.46, m), H-5 [δ_H = 1.75 (m), 1.65 (m)] in the TOCSY spectrum. Its ROESY spectrum suggested that H-8, H-11, H-12, H-13 and H-18 were β-orientated due to correlations [H-11 (δ_H = 4.70, t, *J* = 3.7 Hz) with H-13 (δ_H = 3.04, m), H-12 (δ_H = 2.47, m), and H-17 (δ_H = 1.02, t, *J* = 7.5 Hz); H-8 with H-16 (δ_H = 1.68, m); H-12 with H-18 (δ_H = 4.58, m)] and because of the 10-ethyl β-orientation in *Stemona*-type alkaloids. The α-orientation of H-1 was proposed based on ROESY cross-peaks: H-12/H-2β (δ_H = 1.78, m), H-2α (δ_H = 2.15, m)/H-1(δ_H = 2.85, m). Moreover, ROESY correlations [H-18/H-12 and H-20 (δ_H = 2.73, m), H-20/H-19β (δ_H = 2.00, m), H-19α (2.50, m)/H-3 (4.53, m)] indicated that the α-methyl-γ-lactone ring was attached to C-3 in a β-orientation together with an α-configuration of Me-20 (Fig. **2** and Table **1**). The full assignments and connectivities were determined by ^{1}H-^{1}H COSY, HSQC, HMBC, TOCSY and HMQC-TOCSY spectra.

Neotuberostemonone (**2**) has a molecular formula of $C_{22}H_{31}NO_6$ as deduced from HR-ESI-MS at *m/z* = 428.2047 [M + Na]$^+$ (calcd.: 428.2049). The characteristic cleavage fragment *m/z* = 306 [M – $C_5H_7O_2$]$^+$ in the EI-MS indicated that **3** has an α-methyl-γ-lactone ring connected to C-3 of the A ring [9]. The ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of tuberostemonone except for the chemical shifts of rings A, B and C [10], which suggested that the stereo-configurations of the compounds were different. ROESY correlations in **2** among H-11 (δ_H = 4.54, t, *J* = 3.5 Hz), H-12 (δ_H = 3.56, m), H-13 (δ_H = 2.81, m), H-17 (δ_H = 0.96, t, *J* = 7.5 Hz), as well as H-9 (δ_H = 2.74, m) indicated that H-9, H-11, H-12 and H-13 are all β-oriented. ROESY correlations from H-19β to H-11 and H-20, from H-19α to H-22, from H-18 to H-2β, H-20, and H-5β, from H-2α (δ_H = 2.04, m) to H-3 (δ_H = 4.85, m) indicated that H-3 and Me-20 were α-orientated (Fig. **2**).

Compound **3** was found to possess a molecular formula of $C_{22}H_{29}NO_7$ based on the HR-ESI-MS data (*m/z* = 442.1835 [M + Na]$^+$, calcd.: 442.1841). The ^{1}H-NMR, ^{13}C-NMR and DEPT spectra

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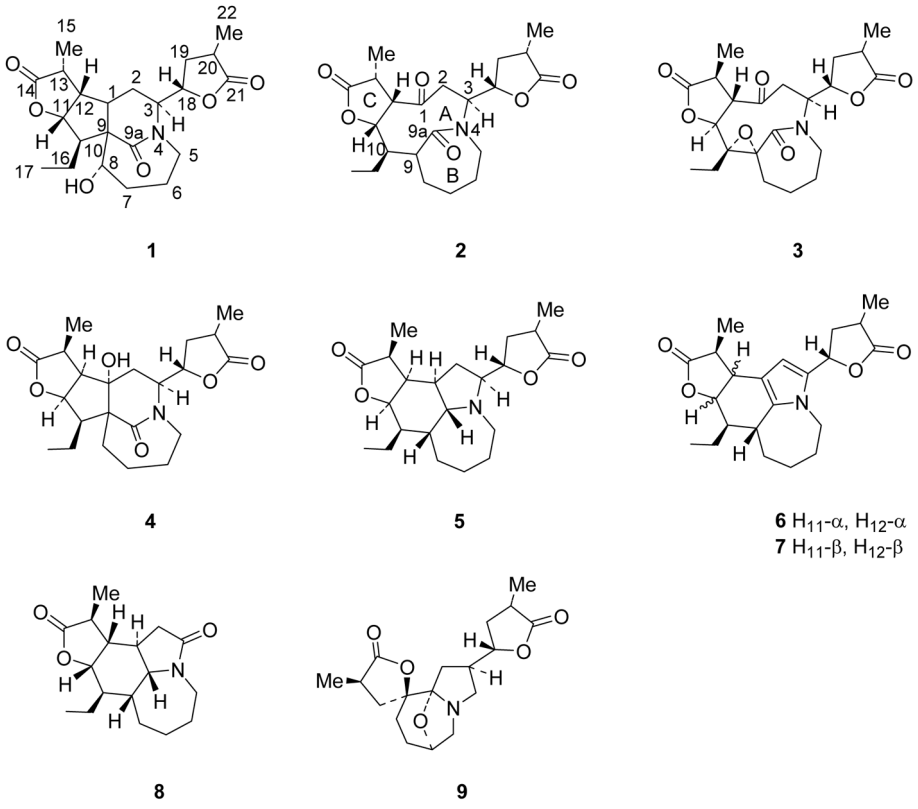


1 **2** **3**

4 **5**

6 H_{11}-α, H_{12}-α
7 H_{11}-β, H_{12}-β

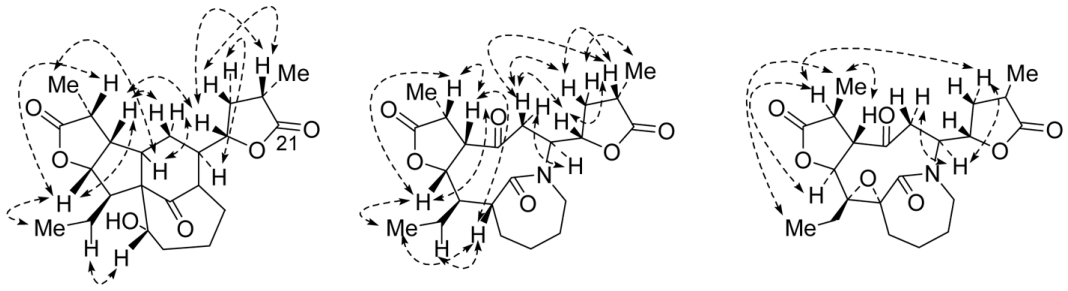
8 **9**

Fig. **1** Structures of alkaloids from Stemona *mairei*.

Table **1** NMR spectral data for **1** – **3** (**1** in acetone-d_6, **2** and **3** in $CDCl_3$, δ in ppm, and J in Hz)

H	δ_H			δ_C		
	1	*2*	*3*	*1*	*2*	*3*
1	2.85 (1H, m)			43.9 d	210.0 s	210.3 s
2α 2β	2.15 (1H, m) 1.78 (1H, m)	2.04 (1H, m) 3.38 (1H, m)	2.43 (1H, m) 3.06 (1H, m)	32.0 t	45.5 t	43.7 t
3	4.53 (1H, m)	4.85 (1H, m)	5.21 (1H, m)	74.2 d	57.5 d	54.6 d
5α 5β	1.65 (1H, m) 1.75 (1H, m)	3.18 (1H, m) 3.43 (1H, m)	3.58 (1H, m) 3.94 (1H, m)	31.1 t	42.4 t	41.9 t
6	1.46 (2H, m)	1.82 (1H, m)	1.70 (2H, m)	20.2 t	27.1 t	26.0 t
7α 7β	1.92 (1H, m) 1.68 (1H, m)	1.77 (1H, m) 1.15 (1H, m)	1.92 (1H, m) 1.70 (1H, m)	31.8 t	23.8 t	20.9 t
8	4.06(1H, br.s)	1.54 (2H, m)	1.67 (2H, m)	77.0 d	27.4 t	26.8 t
9		2.74 (1H, m)		51.8 s	42.0 d	66.1 s
9a				186.5 s	181.5 s	171.1 s
10	1.95 (1H, m)	2.39 (1H, m)		44.7 d	44.8 d	68.5 s
11	4.70 (1H, t, 3.7)	4.54 (1H, t, 3.5)	4.26 1H, d, 7.6)	78.4 d	83.9 d	83.6 d
12	2.47 (1H, m)	3.56 (1H, m)	3.21 (1H, t, 7.6)	50.3 d	58.6 d	41.9 d
13	3.04 (1H, m)	2.81 (1H, m)	2.79 (1H, m)	42.5 d	43.1 d	57.4 d
14				178.5 s	178.1 s	176.2 s
15	1.18 (1H, d, 7.5)	1.02 (1H, d, 7.5)	1.33 (1H, d, 7.5)	11.0 q	12.0 q	16.1 q
16	1.68 (2H, m)	1.63 (1H, m) 1.55 (1H, m)	2.19 (1H, m) 1.43 (1H, m)	18.4 t	20.9 t	20.2 t
17	1.02 (1H, t, 7.5)	0.96 (1H, t, 7.5)	1.04 (1H, t, 7.5)	12.6 q	10.0 q	9.9 q
18	4.58 (1H, m)	4.56 (1H, m)	4.73 (1H, m)	80.9 d	75.2 d	77.9 d
19α 19β	2.50 (1H, m) 2.00 (1H, m)	2.72 (1H, m) 1.54 (1H, m)	1.66 (1H, m) 2.52 (1H, m)	33.6 t	34.7 t	33.6 t
20	2.73 (1H, m)	2.52 (1H, m)	2.81 (1H, m)	35.9 d	35.0 d	35.4 d
21				179.1 s	178.8 s	177.8 s
22	1.15 (1H, d, 7.5)	1.27 (1H, d, 7.5)	1.30 (1H, d, 7.5)	15.5 q	14.8 q	15.1 q

All ^1H- and ^{13}C-NMR were obtained on DRX-500 MHz spectrometers except for the ^{13}C-NMR of **1**, AM-400 MHz.



1 2 3

Fig. **2** Key ROESY correlations of compounds **1** – **3.**

of **3** were similar to those of **2** except for two methines changing to two quaternary carbons [δ_C = 68.0 (s) and 66.1 (s)]. The two quaternary carbons were assigned to a 9,10-epoxide oxygen which was demonstrated by HMBC correlations [C-10 (δ_C = 68.5, s) with H-8 (δ_H = 1.67, m), H-11 (δ_H = 4.26, d, J = 7.6 Hz), H-12 (δ_H = 3.21, t, J = 7.6 Hz), and H-17 (δ_H = 1.04, t, J = 7.5 Hz); C-9 (δ_C = 66.1, s) with H-7 (δ_H = 1.92 and 1.70, m) and H-16 (δ_H = 2.19 and 1.43, m] (Table **1**). The ROESY correlations of **3** [H-15 (δ_H = 1.33, d, J = 7.5 Hz)/H-12 and H-17, H-11/H-13 (δ_H = 2.79, m)] and the fact that no correlation between H-11 and H-12 was observed indicated that H-12 and H-15 were both β-oriented, while H-11, H-13, and the 9,10-epoxide oxygen possessed α-orientations. In addition, the relative configuration of H-3 was deduced as α based on the finding that H-19α (δ_H = 1.66, m) correlated with H-3 (δ_H = 5.21, m) and H-13 in the ROESY spectrum (Fig. **2**).

Materials and Methods

General: All the melting points were obtained on an XRC-1 micromelting apparatus and are uncorrected. IR (KBr) spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer (Bio-Rad; Hercules, CA, USA). The NMR spectra (^{1}H, ^{13}C, DEPT, ROESY, ^{1}H-^{1}H COSY, HMQC and HMBC) were obtained on Bruker AM-400 and DRX-500 MHz spectrometers (Bruker; Fällanden, Switzerland) with TMS as an internal standard. MS data were obtained on a VG Autospec-3000 spectrometer (VG; Manchester; UK). Column chromatography was performed with silica gel (Qingdao Meigao Chemical Group Co. Ltd; Qingdao, China). TLC was performed on silica gel GF254 plates, and spots were detected by spraying with Dragendorf's reagent.

Plant material: The roots of *S. mairei* were collected from Lijiang, Yunnan province, People's Republic of China. A voucher specimen (No. 0311006) has been deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, the Chinese Academy of Sciences.

Extraction and isolation: Dried roots of *S. mairei* (9.0 kg) were cut and refluxed with 95% EtOH (3×15 L). After removal of the EtOH under reduced pressure, the viscous concentrate was acidified with dilute (2%) HCl and then partitioned with $CHCl_3$ (3×3 L). The water layer was adjusted to pH = 9 – 10 with NaOH solution and extracted with $CHCl_3$ (3×3 L) again. Crude **5** was crystallized from the concentrated $CHCl_3$ solution and further recrystallized from the MeOH solution. The mother liquor was concentrated under vacuum to yield 45 g of residue, of which 40 g were absorbed by silica gel (50 g) and subjected to column chromatography over silica gel (5×100 cm, 500 g), eluting with petroleum ether-Me_2CO [9:1 (6 L), 8:2 (9 L), 7:3 (6 L), 6:4 (5 L), 1:2 (8 L)] to give 4 fractions (I – IV). Fraction II (14 g) was purified by column chromatography over silica gel (4×75 cm, 300 g) developed with petroleum ether-Me_2CO [9:1 (5 L), 17:3 (4 L), 8:2 (5 L)] to afford compound **5** (50 mg), 2-oxostenine (6 mg) and a mixture of **6** and **7** (100 mg). Fraction III (7 g) was loaded on CC over silica gel (3×60 cm, 100 g) to give subfractions A and B using petroleum ether-Me_2CO [7:3 (4 L)]. Subfraction A (3.2 g) was further chromatographed on RP-18 (40 – 63 μm, 3×45 cm) eluted with MeOH-H_2O [7:3 (1.2 L), 8:2 (1.6 L)] to afford compounds **1** (7 mg) and **3** (11 mg), respectively. Compound **8** (23 mg) was obtained from fsubraction B (1.4 g) also by RP-18 with MeOH-H_2O. Fraction IV (6 g) was also subjected to RP-18 silica gel (40 – 63 μm, 3×45 cm) to yield compounds **4** (5 mg) and **2** (23 mg) using MeOH-H_2O [6:4 (1.5 L), 7:3 (1.5 L), 8:2 (2.0 L)] as eluent.

Maireistemoninol (**1**): $C_{22}H_{31}NO_6$, white needle crystals, m.p. 155 – 157 °C; $[\alpha]_D^{24}$: 98.48° (*c* 0.24, $CHCl_3$); IR (KBr): ν_{max} = 3477, 1762, 1760, 1669 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table **1**; EI-MS: *m/z* = 406[M + H]$^+$ (1), 389 (5), 371 (10), 360 (100), 342 (45), 332 (80), 290 (55), 262 (75); HR-ESI-MS: *m/z* = 428.1599 [M + Na]$^+$ (calcd. for $C_{22}H_{31}NO_6Na$: 428.2049).

Neotuberostemonone (**2**): $C_{22}H_{31}NO_6$, white square crystals, m.p. 228 – 230 °C; $[\alpha]_D^{24}$: 129.63° (*c* 0.38, $CHCl_3$); IR (KBr): ν_{max} = 1779, 1776, 1691, 1640 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table **1**; EI-MS: *m/z* = 405 [M]$^+$ (4), 306 [M – $C_5H_7O_2$]$^+$ (100); HR-ESI-MS: *m/z* = 428.2047 [M + Na]$^+$ (calcd. for $C_{22}H_{31}NO_6Na$: 428.2049).

Epoxytuberostemonone (**3**): $C_{22}H_{29}NO_7$, white needle crystals, m.p. 286 – 288 °C; $[\alpha]_D^{24}$: –67.53° (*c* 0.16, $CHCl_3$); IR (KBr): ν_{max} = 3434, 1780, 1715, 1664 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table **1**; EI-MS: *m/z* = 419 [M]$^+$ (65), 363 (10), 320 [M – $C_5H_7O_2$]$^+$ (25), 292 (30), 252 (50); HR-ESI-MS: *m/z* = 442.1835 [M + Na]$^+$ (calcd. for $C_{22}H_{29}NO_7Na$: 442.1841).

Supporting information

1D and 2D-NMR spectra of the known compounds are available as Supporting Information.

Acknowledgements

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