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Two New Alkaloids from Roots of *Stemona tuberosa*

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Abstract: The roots of *Stemona tuberosa* have been used as an antitussive and insecticide remedy for thousands of years. On the chemical investigation of the roots of *S. tuberosa*, two new tuberostemonine-type alkaloids, epoxy-tuberostemonol (**1**), and neotuberostemoenone (**2**), together with a known alkaloid, neotuberostemonine, were isolated and identified. The structures of new alkaloids were established on the basis of 1D and 2D NMR and other spectroscopic analyses.

Keywords: *Stemona tuberosa*; tuberostemonine; alkaloids; NMR. © 2014 ACG Publications. All rights reserved.

1. Introduction

The herb Radix Stemonae ('Baibu' in Chinese) has been used as an antitussive and insecticide remedy for thousands of years [1–2]. The species, *Stemona tuberosa* (Stemonaceae), is prescribed in the Chinese Pharmacopoeia as one of three authentic plant resources for Baibu [1]. More than 60 alkaloids have been isolated from *S. tuberosa* of different localities [3–4], which can be classified into tuberostemonine-type [5–8], stemoninine-type [9–10] and croomine-type [8, 11–12]. Considering the great variation of chemical constitute of this species, thoroughly investigation of the minor constituents is needed. Our previous paper has reported seven new alkaloids from *S. tuberosa* collected from Yunnan province, southwest of China [8].

2. Materials and Methods

2.1. Plant Source

The plant material was collected from Wenshan county, Yunnan province on May 2005 and identified by associate professor *Jin-gui Shen*. A voucher specimen (No. SIMM-YYE-05-401) was deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

2.2. Extraction and Isolation

The air-dried roots of *S. tuberosa* were ground into powder and extracted with 95% ethanol. The crude extract was acidified with dilute HCl (4%) to pH 1-2 and partitioned between CH_2Cl_2 and water. The aqueous part was then basified with aqueous NH_3 (28%) to pH 9-10 and extracted with CH_2Cl_2 to afford 82 g crude alkaloids. 70 g crude alkaloids were subjected to column chromatography over Silica

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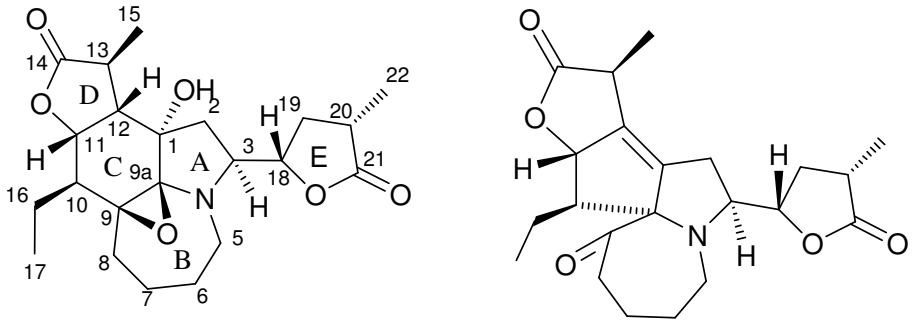
gel and eluted gradient with petroleum ether-acetone from 8:1 to 1:2 to yield 12 fractions. Fraction 5 (850 mg) was subjected to column chromatography over silica gel with petroleum-acetone (4:1→2:1) to obtain neotuberostemonine, epoxy-tuberostemonol (**1**) and neotuberostemoenone (**2**).



1 **2**

Figure 1. Structures of epoxy-tuberostemonol (**1**) and neotuberostemoenone (**2**) isolated from *S. tuberosa.*

3. Results and Discussion

3.1. Results

Epoxy-tuberostemonol (**1**) was obtained as white amorphous powder. Its HR-EI-MS showed the molecular ion peak at *m/z* 405.2150 $[M]^+$, indicating the molecular formula of **1** as $C_{22}H_{31}NO_6$ (calc. 405.2149). Eight degrees of unsaturation were determined for **1**. The broad and sharp IR absorption at 3475 cm^{-1} indicated the existence of a hydroxyl group. The sharp absorption band at 1761 cm^{-1}, together with the peak at *m/z* 306 ($[M\text{-}C_5H_7O_2]^+$) in the EI-MS spectrum, suggested the presence of a typical α-methyl-γ-lactone moiety in *Stemona* alkaloids [3-4]. The ^{1}H-NMR spectrum (Table 1) displayed signals of three methyl groups (δ 1.05 (*t*, *J* = 7.5 Hz), 1.28 (*d*, *J* = 7.3 Hz), 1.39 (*d*, *J* = 7.8 Hz)), and two methine protons bearing oxygen-atom (δ 4.15 (*m*), 4.36 (*dd*, *J* = 7.5, 9.5 Hz)). The ^{13}C-NMR and DEPT spectra (Table 1) showed 22 resonances, which were classified into two carbonyl carbons, three sp^3 quaternary carbons, seven sp^3 tertiary carbons, seven sp^3 secondary carbons and three sp^3 methyl carbons. Thus one active proton, or a hydroxyl group, was revealed in the molecule. Because two of eight unsaturation degrees were ascribed to the carbonyl groups, the left six allowed a hexacyclic skeleton in the molecule of **1**.

Three structural fragments, C(2)-C(3)-C(18)-C(19)-C(20)-Me(22), C(5)-C(6)-C(7)-C(8), and Me(17)-C(16)-C(10)-C(11)-C(12)-C(13)-Me(15) (bold lines in Figure 2a) were established on the basis of the ^{1}H,^{1}H-COSY, HSQC and HMBC spectra. The above data suggested compound **1** contains tuberostemonine-type pentacyclic skeleton [8]. The peak at δ 80.1 was determined to be at position 1 by HMBC cross-peaks from H-C(11) (δ 4.36 (*dd*, *J* = 7.5, 9.5)) and H-C(13) (δ 2.82 (*dq*, *J* = 1.4, 7.3)) to its quaternary carbon atom. Additionally the HMBC correlation between the active proton and C(1) assigned the hydroxyl group at position 1. This structural moiety is similar with that of neotuberostemonol [7]. Compared with their ^{1}H and ^{13}C NMR spectra, two low-field quaternary carbons in **1** were identified instead of two olefinic carbons at C(9) and C(9a) in neotuberostemonol . The more low-field quaternary carbon (δ 106.4) was assigned as C(9a) according to the HMBC correlations between H-C(2a) (δ 1.68 (*m*)), H-C(3) (δ 3.10 (*m*)), H-C(5b) (δ 2.88 (*d*, *J* = 14.6)), H-C(12) (δ 2.16 (*dd*, *J* = 1.4, 4.3)) and this carbon. The HMBC cross peaks between H-C(16) (δ 1.85 (*m*) and 1.52 (*m*)) and the remained carbon caused to assign it as C(9). Considering the molecular formula and unsaturation degree, the remained oxygen atom was deduced to conjoin C(9) and C(9a) to form an epoxy bond, which might generate the last ring and accounted for the remained one unsaturation . Compared the NMR spectra of stemona-amide [14], the low-field shifts of H-C(10) and C(9a), and high-field shifts of C(1), H-C(11) and H-C(12) further supported that epoxy ring located at C(9) and C(9a), and hydroxyl group at C(1) in compound **1**. Accordingly the planner structure was established for compound **1**, which was the same as that of tuberostemonoxirine [15].

The relative configuration of **1** was fixed by the ROESY spectrum (broken arrows in Figure 2b) and its biogenesis. Till now, almost all the reported tuberostemonine-type alkaloids possess *β*-oriented ethyl group at C(10) [5–8]. The correlations of H-C(11)/H-C(12), H-C(11)/Me-15, H-C(11)/Me-17 and H-C(12)/Me-15 indicated H-C(11), H-C(12), Me-15 and Me-17 were at the same side and *β*-oriented. The cross-peak of H-C(12)/H*b*-C(2) suggested the hydroxyl was *α*-face and the relative configuration of C(1) was *S**. The epoxy ring was inferred to be *β*-oriented according to its biogenetic pathway. Compared with tuberostemonoxirine [15], compound **1** has different configurations at C(1), C(9), C(9a), C(10), C(11), C(12) and C(13). Accordingly, the structure of **1** was fully constructed and the detailed assignments of the ^{1}H- and ^{13}C-NMR signals (Table 1) were determined by HSQC, HMBC and ^{1}H-^{1}H COSY spectra.

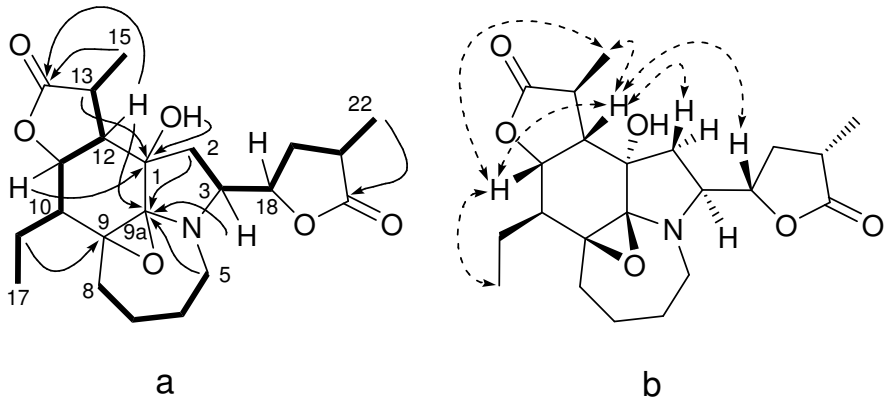


Figure 2. a: ^{1}H-^{1}H correlations (bold lines) and key HMBC correlations (arrows) of **1**. b: Key ROESY correlations (broken arrows) of **1**

Neotuberostemoenone (**2**) was obtained as a colorless solid. The molecular formula of **2** was determined as $C_{22}H_{29}NO_5$ by its HR-ESI-MS (*m/z* 388.2051 $[M+1]^+$; calc. 388.2046), together with ^{1}H- and ^{13}C-NMR spectra. The strong and sharp IR band at 1771 cm^{-1} indicated the existence of typical γ-lactone. The fragment ion at *m/z* 288 $[M-C_5H_7O_2]^+$ indicated the presence of an α-methyl-γ-lactone ring annexed to C(3). The ^{1}H-NMR spectrum (Table 1) displayed signals of two *doublet* methyl groups (*δ* 1.22 (*d*, *J* = 6.9 Hz, Me(22)), 1.24 (*d*, *J* = 8.0 Hz, Me(15))), a triplet methyl group (*δ* 0.87 (*t*, *J* = 7.5 Hz, Me(17))), and four down-field protons (*δ* 3.58 (*ddd*, *J* = 2.5, 5.0, 13.5 Hz, H*b*-C(5)), 3.73 (*ddd*, *J* = 5.0, 5.5, 7.0 Hz, H-C(3)), 4.35 (*ddd*, *J* = 5.5, 6.5, 12.0 Hz, H-C(18)), 4.87 (*d*, *J* = 1.0 Hz, H-C(11))). The ^{13}C-NMR and DEPT spectra (Table 1) indicated 22 carbon signals including three methyl carbons, seven methylene carbons, six methine carbons, one quaternary *sp*^{3} carbons, two olefinic carbons, two lactone carbonyl carbons, and one ketone carbonyl carbon. The above MS, IR and NMR data were quite similar with those of tuberostemoenone [16-17]. However, difference was found in their optical rotations. The $[\alpha]_D^{20}$ value of compound **2** is -32, while that of tuberostemoenone is +18. The relative configuration of **2** was inferred by the ROESY experiment. The correlations of H-C(11)/Me(15), H-C(12)/Me(15) and H-C(11)/Me(17) indicated that Me(15) was *β*-oriented, which was opposite with that of tuberostemoenone. Thus the structure of **2** was established and named as neotuberostemoenone. The detailed assignments of the ^{1}H- and ^{13}C-NMR signals were shown in Table 1.

3.2. Discussion

In this paper we reported two highly oxidized alkaloids from *S. tuberosa*. The co-occurrences of tuberostemonine, epoxy-tuberostemonol (**1**), and tuberostemoline in the species *S. tuberosa* suggested their possible biogenesis pathway. Tuberostemonine was the original form, which could hydroxylize and dehydrogenate to yield intermediate product A. Then the double bond in intermediate product A could epoxide to form epoxy-tuberostemonol (**1**), which was further oxidized to tuberostemoline (Figure 3).

In our investigation of *Stemona* alkaloids, some plant material only contained one major alkaloid, always original form, but others contained many highly oxidized alkaloids, such as in this sample. The

possibility might be collection season, growing environment, transport, and storage. Especially *S. tuberosa*, the distribution pattern of tuberostemonine-type, stemoninine-type and croomine-type alkaloids is complicated. In our survey of the species in Yunnan province, the components of *S. tuberosa* growing in south or north side of a hill were quite different (data not published). The chemical diversity of *Stemona* alkaloids indicated potential physiological function in plant and also supplied challenging targets for organic synthesis.

Table 1. ^{1}H-NMR and ^{13}C-NMR data for compounds **1** and **2** in $CDCl_3$ [a]

	1		**2**	
	δ (H)	δ(C)[b]	δ (H)	δ(C)
1	2.70 (*s*, OH)	80.1		138.6
2 *a*	1.98 (*m*)	37.6	2.60 (*m*)	40.3
2 *b*	1.68 (*m*)		2.33 (*m*)	
3	3.10 (*m*)	63.9	3.73 (*ddd*, *J* = 5.0, 5,5, 7.0)	61.0
5*a*	3.07 (*dd*, *J* = 13.1, 14.6)	48.2	3.58 (*ddd*, *J* = 2.5, 5.0, 13.5)	53.5
5*b*	2.88 (*d*, *J* = 14.6)		2.82 (*ddd*, *J* = 2.0, 11.5, 13.5)	
6	1.60 (*m*)	30.3	1.83 (*m*)	27.3
6	1.41 (*m*)		1.65 (*m*)	
7	1.80 (*m*)	22.1	1.89 (*m*, 2H)	31.3
7	1.62 (*m*)			
8	2.03 (*m*)	30.4	2.08 (*ddd*, *J* = 2.4, 2.4, 15.0)	25.5
8	1.22 (*m*)		1.98 (*ddd*, *J* = 2.0, 14.0, 15.0)	
9		86.5		203.8
9a		106.4		68.6
10	2.72 (*m*)	46.1	2.62 (*m*)	55.3
11	4.36 (*dd*, *J* = 7.5, 9.5)	82.8	4.87 (*d*, *J* = 1.0)	83.0
12	2.16 (*dd*, *J* = 1.4, 4.3)	48.1		125.8
13	2.82 (*dq*, *J* = 1.4, 7.3)	36.3	2.87 (*q*, *J* = 8.0)	42.6
14		179.5		177.4
15	1.39 (*d*, *J* = 7.8)	18.5	1.24 (*d*, *J* = 8.0)	14.3
16	1.85 (*m*)	23.5	1.60 (*m*)	24.4
16	1.52 (*m*)		1.09 (*m*)	
17	1.05 (*t*, *J* = 7.5)	13.9	0.87 (*t*, *J* = 7.5)	11.0
18	4.15 (*m*)	82.1	4.35 (*ddd*, *J* = 5.5, 6.5, 12.0)	78.9
19*a*	1.53 (*m*)	34.6	2.37 (*m*)	33.7
19*b*	2.40 (*m*)		1.56 (*m*)	
20	2.62 (*m*)	35.1	2.65 (*m*)	35.2
21		178.8		178.2
22	1.28 (*d*, *J* = 7.3)	15.0	1.22 (*d*, *J* = 6.9)	14.8

[a] Bruker DRX 400 MHz spectrometers, chemical shits (ppm) referred to $CDCl_3$ (7.26).
[b] By DEPT sequence.

Neotuberostemonine was obtained as white amorphous powder and its complete structural assignment was carried out by comparing with the related reference [18].

Epoxy-tuberostemonol (**1**): White amorphous powder. $[\alpha]_D^{20}$ -16 (*c* = 0.10, $CHCl_3$). IR ν_{max} (KBr): 3475, 2943, 1761, 1458, 1203, 1020, 912 cm^{-1}. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: *m/z* 405 (2), 364 (100), 306 (29, $(M\text{-}C_5H_7O_2)^+$), 278 (38). HR-EI-MS: *m/z* 405.2150 ($C_{22}H_{31}NO_6^+$; calc. 405.2151).

Neotuberostemoenone (**2**): Colorless solid. $[\alpha]_D^{20}$ -32 (*c* = 0.07, $CHCl_3$). IR ν_{max} (KBr): 2935, 1771, 1167, 1010, 924 cm^{-1}. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS: *m/z* 388.3 $[M+1]^+$, 410.2 $[M+Na]^+$, 386.3 $[M-1]^-$. HR-ESI-MS: *m/z* 388.2051 ($C_{22}H_{30}NO_5$; cacl. 388.2046).

Neotuberostemonine: White amorphous powder. EIMS *m/z* 375 (*M*), 276 (base peak, $[M\text{-}C_5H_7O_2]$), 232, 174, 149,128. ^{1}H-NMR (CDCl3): 1.00 (3H, *t, J* = 7.2 Hz, H-17), 1.25 (3H, *d*, *J* = 7.0 Hz, H-15), 1.28 (3H, *d*, *J* = 7.0 Hz, H-22), 1.75 (1H, *m*, H-10), 2.88 (1H, *m*, H-13), 4,47 (1H, *m*, H-18), 4.51 (1H, *t*, *J* = 3.0 Hz, H-11). It was identified as neotuberostemonine [18].

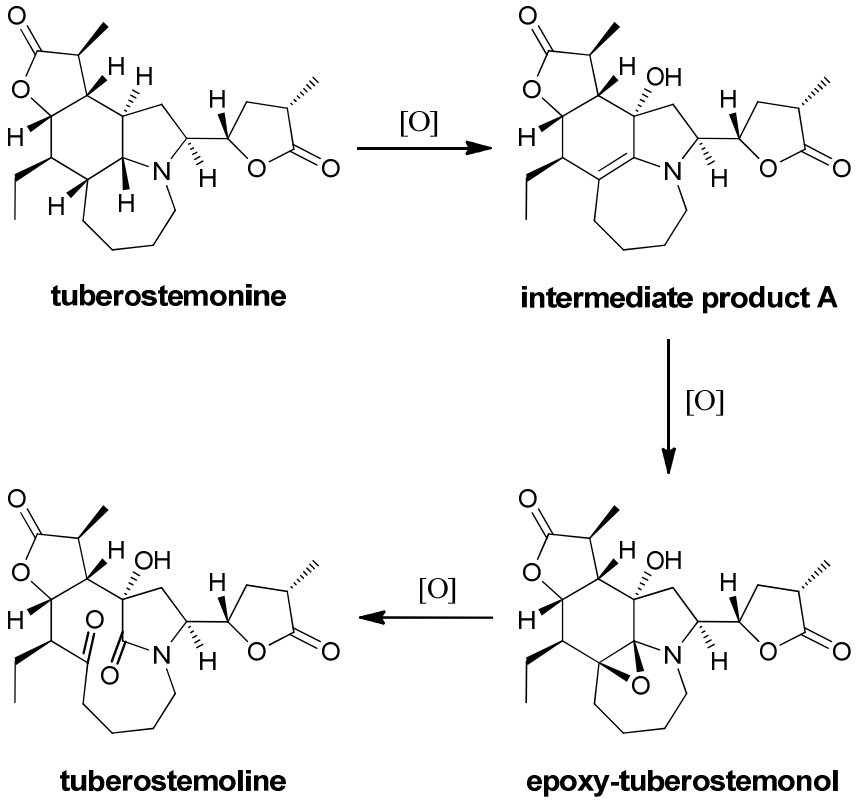


Figure 3. Proposed biogenesis pathway of epoxy-tuberostemonol.

Acknowledgments

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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