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Tuberostemonine O from the Roots of *Stemona tuberosa*

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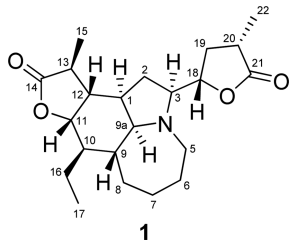
Stemonae Radix is the dried root of *Stemona* species (Stemonaceae) including *S. tuberosa* Lour, *S. japonica* (Blume) Miquel, and *S. sessilifolia* (Miquel) Miquel. Stemonae Radix is a traditional medicine used as antitussive and anti-helminthic drugs in Asia.[1] Previous phytochemical studies on *Stemona* species have reported on the isolation of various types of alkaloids including croomine, protostemonine, stemoninine, maistemonine, tuberostemonine, and neotuberostemonine and led to a chemotaxonomic conclusion that each different characteristic alkaloids were distributed within each specific *Stemona* species.[2-4] There have been several reports that *S. tuberosa* containes stichoneurine- and croomine-types alkaloids such as tuberostemonine, neotuberostemonine, and stemoninine, while non-tuberosa group contains protostemonine type alkaloids.[2-5] Thus, Stemonae Radix used in the present study was identified as *S. tuberosa* since the stereoisomers of tuberostemonine were isolated in this study. The stereoisomers of tuberostemonine such as tuberostemonine A to N, which have differences in their relative configurations, have been identified as new constituents of *S. tuberosa* in its previous phytochemical studies.[3,7,9,11] In the present study, a new tuberostemonine, (2*S**,7a*R**,8*R**,8a*S**,11*S**,11a*S**,11b*R**,11c*S**)-8-ethyldodecahydro-11-methyl-2-[(2*S**,4*S**)-tetrahydro-4-methyl-5-oxo-2-furanyl]-furo[2,3-*h*]pyrrolo[3,2,1-*jk*][1]benz-azepin-10(2*H*)-one (tuberostemonine O) (1) (Figure 1) was isolated from the roots of *S. tuberosa*. The structure elucidation of compound **1** is described herein.

Compound **1** was obtained as light yellow powder and assigned the protonated molecular formula of $C_{22}H_{34}NO_4$ from its HRESIMS (*m/z* 376.2483) $[M+H]^+$. In the ^{1}H-NMR spectrum, a primary methyl group appeared at δ_H 0.95 (3H, t, J = 7.4 Hz, H-17) and the secondary methyl groups were observed at δ_H 1.28 (3H, d, J = 7.2 Hz, H-22) and 1.32 (3H, d, J = 7.6 Hz, H-15). Two low field geminal protons appeared at δ_H 2.29 (1H, m, H-5α) and 3.31 (1H, dt, J = 13.4, 3.6 Hz, H-5β) and two protons at the oxygenated carbons were shown at δ_H 4.31 (1H, m, H-18) and 4.33 (1H, m, H-11). The ^{13}C- and DEPT NMR spectra showed 22 skeletal carbon signals including three methyls, seven methylenes, ten methines, and two carbonyls. The above characteristic data suggested that **1** is related structurally to tuberostemonine (Table 1).[8] The spinning COSY correlations of H-11→H-12→H-13→H_3-15 and H-18→H-19→H-20→H_3-22 with two oxomethines [δ_H 4.31(H-18)/δ_C 82.1(C-18) and 4.33 (H-11)/ 83.4(C-11)] as starting points and the HMBC correlations of H_3-15/C-14, H-13/C-14, H_3-22/C-21, and H-20/C-21 provided evidence for two α-methyl-γ-lactone rings.[9] The COSY spectrum of **1** exhibited spin systems of H-5→H-6→H-7→H-8→H-9→H-9a and H-9a→H-1→H-2→H-3 involving typical three low field protons attached to the N-atom at [δ_H 2.29 and 3.31 (each H-5), 2.83 (H-3), and 3.00 (H-9a)], indicating the presence of the nitrogen fused perhydroazaazulene ring.[12] Additional COSY correlations of H-11→H-10→H_2-16→H_3-17 and the HMBC correlations of H-13/C-1, H-11/C-1, H-10/C-9, H_2-16/C-9, H_2-2/C-18, and H-3/C-18 allowed to confirm the connectivities of the above subgroups. Further analysis of the ^{1}H-^{1}H COSY, HSQC, and HMBC data of **1** (Figure 2) allowed unambiguous assignments for all of the ^{1}H- and ^{13}C-NMR signals of **1**. To determine the relative configurations of **1**, the analysis of ^{1}H-^{1}H NOESY experiments was applied.[3,7-11] All the NOE correlations of **1** were comparable to the stereostructure of tuberostemonine except for H-9a as shown in Figure 3. The NOESY of **1** exhibited the correlations of H-9a/H-3,H-10, indicating that these three protons had α-orientations. The H-9a in the structure of tuberostemonine was β,[8] which was different with that of **1**. The analysis of NOE correlations allowed the relative configurations of all chiral centers as *rel*-(1*R*, 3*S*, 9*R*, 9a*S*, 10*R*, 11*S*, 12*S*, 13*S*, 18*S*, and 20*S*). Thus, compound **1** was determined as a new alkaloid, namely, tuberostemonine O (IUPAC name : (2*S**,7a*R**,8*R**,8a*S**,11*S**,11a*S**,11b*R**,11c*S**)-8-ethyldodecahydro-11-methyl-2-[(2*S**,4*S**)-tetrahydro-4-methyl-5-oxo-2-furanyl]-furo[2,3-*h*]pyrrolo[3,2,1- *jk*][1]benz-azepin-10(2*H*)-one).

On the other hand, the known alkaloid, tuberostemonine N was isolated as a bulk (3.8 g, 0.04% w/w) from the plant sample used in the present study. This result supported the



1

Figure 1. Chemical structures of **1** isolated from *S. tuberosa*.

Table 1. NMR data for compound **1** and tuberostemonine

No.	**1**[a]		Tuberostemonine[b]	
	δ_H mult., (*J* Hz)	δ_C	δ_H mult., (*J* Hz)	δ_C
1	1.96 m	37.0	1.80 m	41.6
2	2.07 m 0.97 m	29.8	2.18 ddd (12.3, 6.7, 6.7) 1.10 m	32.1
3	2.83 dd (15.6, 7.6)	66.7	3.43 m	65.0
5	3.31 dt (13.4, 3.6) 2.29 m	52.4	3.47 ddd (14.4, 2.8, 1.4) 2.67 ddd (14.4, 10.6, ~1)	48.1
6	1.69 br d (13.4) 1.45 m	30.6	1.53 m 1.44 m	28.1
7	1.97 m 1.13 br d (11.6)	31.5	1.84 m 1.17 m	29.9
8	1.51 m 1.35 m	23.7	1.57 m 1.54 m	30.4
9	1.98 m	39.3	1.82 m	40.7
9a	3.00 dd (9.2, 6.8)	69.8	3.07 dd (11.1, 6.3)	63.6
10	1.55 m	42.9	1.55 m	45.0
11	4.33 m	83.4	4.44 dd (7.8, 7.5)	80.3
12	2.05 m	45.7	2.00 ddd (10.4, 7.5, 7.3)	47.3
13	2.31 m	40.6	2.41 dq (7.3, 7.3)	40.9
14		180.4		179.2[c]
15	1.32 d (7.6)	18.1	1.28 d (7.3)	14.7
16	1.82 m 1.44 m	24.2	1.52 m 1.50 m	24.3
17	0.95 t (7.4)	12.1	0.96 t (7.4)	11.2
18	4.31 m	82.1	4.31 ddd (10.4, 7.6, 5.7)	81.4
19	2.40 ddd (12.8, 8.8, 5.6) 1.56 m	34.5	2.38 ddd (12.4, 8.5, 5.7) 1.47 m	34.6
20	2.64 m	35.1	2.60 ddq (12.4, 8.5, 7.0)	34.8
21		179.6		179.4[c]
22	1.28 d (7.2)	15.2	1.26 d (7.0)	14.9

[a,b]Measured in $CDCl_3$ at 400 MHz (^{1}H-NMR) and 100 MHz (^{13}C-NMR). [b]Taken from the literature.[12] [c]Interchangeable within the column.[12]

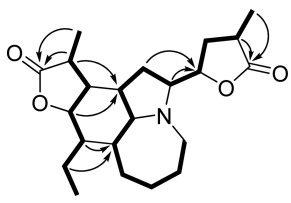


Figure 2. Important ^{1}H-^{1}H COSY (▬) and HMBC (H→C) correlations of **1**.

identification of the plant sample as *S. tuberosa*.

Experimental

General Experimental Procedures. Optical rotation was measured on a P-1010 polarimeter (JASCO, Japan) at 27 °C. IR spectrum was recorded on Nicolet iS10 FT-IR spectrometer (Thermo Fisher Scientific, MA). 1D and 2D NMR experiments were performed on a UNITY INOVA 400 MHz FT-NMR instrument (Varian, CA) with tetramethylsilane (TMS) as internal standard. HREIMS was performed with Waters ACQUITY UPLC system coupled to a Micromass Q-Tof Micro mass spectrometer and Agilent 6220 Accurate-Mass TOF LC/MS system. Silica gel (70-230 mesh and 230-400 mesh, Merck, Germany) was used for column chromatography (CC). Thin-layer chromatographic (TLC) analysis was performed on Kieselgel 60 F 254 plates (silica gel, 0.25 mm layer thickness, Merck, Germany), with visualization under UV light (254 and 365 nm) and Dragendorff's reagent.

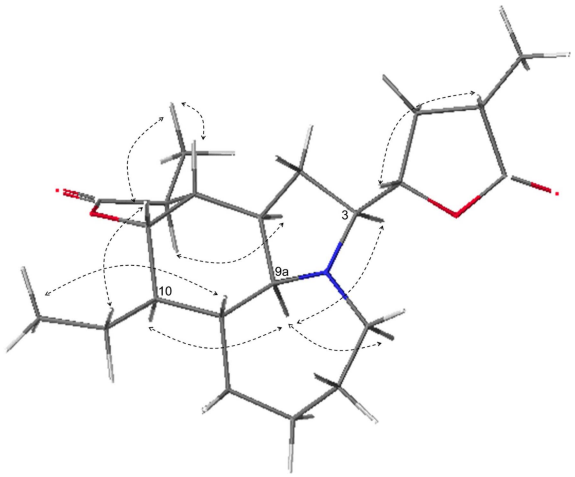


Figure 3. Key NOESY correlations of **1**. The Energy minimized stereostructure of **1** was constructed by MM2 calculation using CAChe 5.0™ molecular modeling program.

Plant Material. The dried roots of *S. tuberosa* were purchased from the Insan Oriental Herbal Market in Seoul, Korea. The sample was identified by Prof. Je-Hyun Lee (College of Oriental Medicine, Dongguk University, Korea). A voucher specimen (No. EA322) has been deposited at the Natural Product Chemistry Laboratory, College of Pharmacy, Ewha Womans University, Korea.

Extraction and Isolation. The roots of *S. tuberosa* (10 kg) were extracted with MeOH (3 × 15 L, 24 h) at room temperature. The solvent was evaporated *in vacuo* to afford a MeOH extract (3.6 kg), which was then suspended in distilled water, and partitioned with *n*-hexane (3 × 5 L), EtOAc (6 × 5 L), and *n*-BuOH (6 × 5 L), successively. The EtOAc-soluble extract (98 g) was separated by silica gel CC (ϕ 10 cm; 70-230 mesh), using gradient mixtures of CH_2Cl_2-MeOH (100:0 → 0:100) as mobile phases, affording 12 fractions (F1-F12). The fraction F6 (3.4 g) was subjected to silica gel CC (ϕ 3 cm; 230-400 mesh) and eluted with gradient solvent systems of *n*-hexane-acetone (5:1 → 1:1) to yield compound **1** (21.8 mg, 0.00023% w/w). The fraction F10 (11.9 g) was chromatographed on silica gel (ϕ 3 cm; 230-400 mesh) with gradient mixtures of *n*-hexane-acetone (9:1 → 2:3) to give tuberostemonine N (3.8 g, 0.04% w/w).

Tuberostemoine O (1): Light yellow powder. $[\alpha]_D^{27}$ +10.9 (*c* 0.09, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 2924, 1770, 1457, 1189, 1018; ^1H- and ^{13}C-NMR data, see Table 1; HRESIMS (positive mode) *m/z* = 376.2483 $[M+H]^+$ (calcd for $C_{22}H_{34}NO_4$: 376.2482).

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Supporting Information. The spectral data of compound **1** are available on request from the correspondence author.

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