# A NEW DITERPENOID ALKALOID, SHARWUPHININE A FROM *DELPHINIUM SHARWURENSE*

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Abstract – A new diterpenoid alkaloid, sharwuphinine A (**1**) was isolated from the aerial part of *Delphinium sharwurense* and the structure possessing a lycoctonine-type C_{19}-diterpenoid alkaloid with a nitrone functionality was elucidated by spectroscopic analysis. Sharwuphinine A (**1**) showed moderate vasorelaxant activity on rat aorta.

Delphinium species have been employed in analgesic balms, sedatives, emetics, and antihelminthics in folk medicine.[1] About twenty species are distributed in Xinjiang Uygur autonomous region, People's Republic of China among approximately 300 species in the world. Diterpenoid alkaloids are the major active constituents of *Delphinium* plants belonging to Ranunculaceae. We have so far isolated several diterpenoid alkaloids such as elatine from *Delphinium sharwurense* W. T. Wang,[2] which showed muscle relaxing activity.[3] On continuing search for chemical constituents of *D. sharwurense* with biological activity, we have isolated a new diterpenoid alkaloid with a nitrone functionality, sharwuphinine A (**1**), which showed moderate vasorelaxant activity against rat aorta. This paper describes the isolation and structure elucidation of **1** and vasodilator effect on rat aorta.

1   **2**   **3**
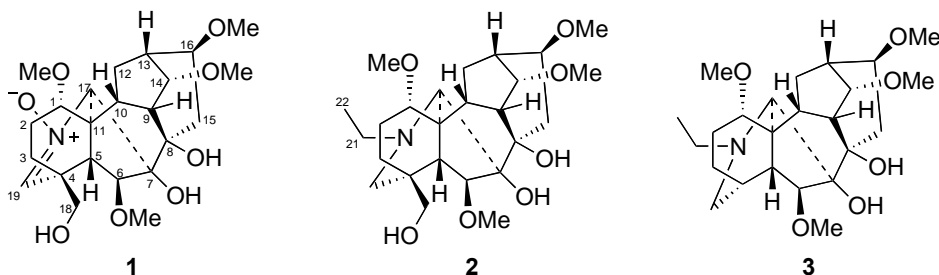
[†]Dedicated to Professor Emeritus Akira Suzuki, Hokkaido University, on the occasion of his 80th birthday.

The aerial parts of *D. sharwurense* were extracted with 80% EtOH, and the extract was in turn partitioned between $CHCl_3$ and 5% H_2SO_4 aq. Water-soluble materials, which were adjusted to pH 10 with saturated Na_2CO_3 aq., were extracted with $CHCl_3$ and the residual materials were extracted with BuOH. BuOH-soluble materials were subjected to a silica gel column ($CHCl_3$/MeOH 1:0→1:1, $CHCl_3$/MeOH/H_2O 5:5:1, and MeOH) and the fractions eluted by $CHCl_3$/MeOH (50:1→5:5) were subjected to an amino silica gel column ($CHCl_3$/MeOH 1:0→1:1) to afford sharwuphinine A (**1**, 0.0004%) together with lycoctonine (**2**, 0.002%)[4] and deoxymethylenelycoctonine (**3**, 0.005%).[5]

Sharwuphinine A **1** $\{[\alpha]_D^{23}$ +25 (*c* 0.2, MeOH)$\}$ showed the pseudomolecular ion peak at *m/z* 454 $(M+H)^+$ in ESIMS, and the molecular formula, $C_{23}H_{35}NO_8$, was established by HRESIMS [*m/z* 454.2446, $(M+H)^+$ Δ +1.1 mDa]. IR spectrum suggested the presence of hydroxy (3433 cm^{-1}) and an imino (1650 cm^{-1}) groups. The ^{13}C NMR spectrum of **1** (Table 1) disclosed twenty-three carbon signals due to four sp^3 quaternary carbons (δ_C 45.7, 53.0, 78.8, and 86.9), one sp^2 methine (δ_C 144.1), nine sp^3 methines (δ_C 39.4, 43.7, 44.4, 46.1, 79.0, 82.1, 84.1, 85.6, and 91.0), five sp^3 methylenes (δ_C 21.0, 26.3, 32.0, 34.3, and 65.0), and four methoxy groups (δ_C 56.6, 57.0, 58.1, and 59.3). Since 1 out of 7 elements of unsaturation implied by the molecular formula was accounted for, **1** was inferred to possess 6 rings.

Four partial structures **a** (C-1 ~ C-3), **b** (C-5 ~ C-6), **c** (C-9 ~ C-14), and **d** (C-15 ~ C-16) were assigned by detailed analyses of 2D NMR data (^1H-^1H COSY and HMQC) of **1**. Connectivity of units **a** and **b** through C-4 and C-11 was implied by HMBC correlations for H$_2$-3 and H-5 to C-4 (δ_C 45.7), and H-1 and H-5 to C-11 (δ_C 53.0). The presence of an imino group at C-19 was verified by the IR absorption (1650 cm^{-1}) and a methine carbon resonance (δ 144.1, C-19). The UV absorption (λ_{max} 250 nm) corroborated the presence of a nitrone group.[6] The HMBC cross-peak of H-19 to C-17 (δ_C 79.0) and H$_2$-18 to C-4 placed the nitrone between C-17 and C-19 and a hydroxymethylene at C-4, respectively. HMBC correlations were observed for H-5 to C-10 (δ_C 43.7), H-6 to C-7 (δ_C 86.9) and C-8 (δ_C 78.8), and H$_2$-15 to C-7, C-8, and C-9 (δ_C 44.4), suggesting that a cyclopentane ring (unit **c**) was connected through C-7, C-8, and C-11 to unit **b** in a decahydroazulene ring system and also a cyclohexane ring was formed with unit **d** through C-8. In addition, connectivities between four methoxy groups and C-1, C-6, C-14, and C-16 were implied by HMBC correlations for each methoxy group to C-1, C-6, C-14, and C-16, respectively. All of this evidence indicated that sharwuphinine A (**1**) is a lycoctonine-type C_{19}-diterpenoid alkaloid and **1** was a nitrone of lycoctonine without N-ethyl group.

The relative stereochemistry of **1** was elucidated by NOESY correlations. It was no doubt that one relative stereostructure existed for fused-tetracyclic ring system (C-1 ~ C-11, C-17, C-19 and N) with no strain, which was satisfied with NOESY correlations of H-5/H-9 and H-19/H-3a and H-6. The NOESY correlation of H-2a/H-5 indicated that the cyclohexane ring (C-1 ~ C-5 and C-11) took boat conformation.

In addition, H-1 was correlated with both H-10 and H-12a, and H-6 with H-19 indicating that H-1 was β-oriented and H-6 was α-oriented. Furthermore, the relative configurations at C-14 and C-16 were deduced from NOESY correlations of H-14/H-10 and H-12a, and those among H-12b, H-16, and H-17 as shown in computer-generated 3D drawing (Figure 2).

Compound **1** was named sharwuphinine A, and the first natural diterpene alkaloid with a nitrone functionality. Biogenetically sharwuphinine A might be derived from lycoctonine by formation of N-oxide followed by N-deethylation and then production of a hydroxylamine derivative.
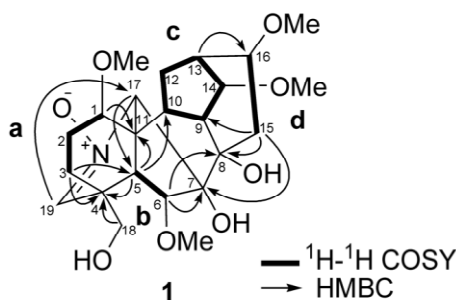

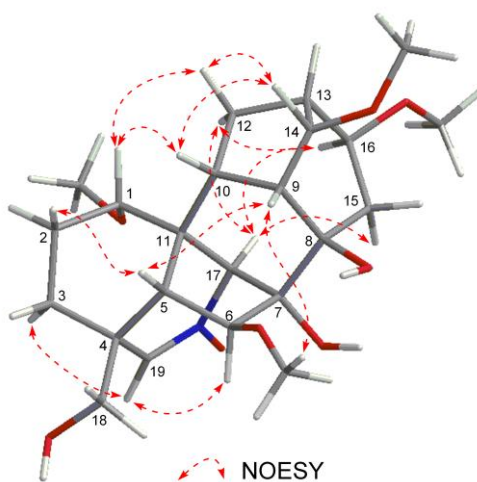Figure 1. Selected 2D NMR correlations of **1**.

Figure 2. Selected NOESY correlations and relative stereochemistry for **1**

The vasodilators are useful for treatment of cerebral vasospasm and hypertension, and for improvement of peripheral circulation.[7] Many natural products evoked vasodilatation through multiple mechanisms such as the nitric oxide system, potassium channel, and calcium channel functions. Among diterepenoid alkaloids, mesaconitine, one of the aconite alkaloids, has been inhibited against phenylephrine-induced contraction in the endothelium-intact, but not endothelium-denuded, aortic rings.[8] When phenylephrine (PE) 3×10^{-7} M was applied to thoracic aortic rings with endothelium after achieving a maximal response,

we added sharwuphinine A (**1**), lycoctonine (**2**), and deoxymethylenelycoctonine (**3**). Among them, sharwuphinine A (**1**) showed a moderate vasorelaxant activity (40% relaxation at 10^{-4} M), whereas lycoctonine (**2**) and deoxymethylenelycoctonine (**3**) did not show vasorelaxant actions. Vasodilation may seem to be influenced by the hydrophobicity of a nitrogen atom. Efforts are currently underway to determine the mode of action of sharwuphinine A (**1**) on vasorelaxant activity.

Table 1. ^{1}H and ^{13}C NMR Data [δ_H (J, Hz) and δ_C] of sharwuphinine A (**1**) in CD$_3$OD at 600MHz

Position	δ_H	δ_C
1	3.43 (1H, brs)	82.1
2a	1.40 (1H, m)	21.0
2b	1.90 (1H, m)	
3a	1.62 (1H, m)	26.3
3b	1.95 (1H, dd, 14.0, 7.6)	
4		45.7
5	2.10 (1H, brs)	46.1
6	3.93 (1H, brs)	91.0
7		86.9
8		78.8
9	2.87 (1H, dd, 6.2, 4.8)	44.4
10	2.21 (1H, m)	43.7
11		53.0
12a	1.30 (1H, dd, 13.7, 4.1)	32.0
12b	2.18 (1H, m)	
13	2.39 (1H, dd, 6.5, 4.4)	39.4
14	3.72 (1H, dd, 4.8, 4.4)	85.6
15a	1.66 (1H, dd, 15.1, 8.1)	34.3
15b	2.83 (1H, dd, 15.1, 8.5)	
16	3.26 (1H, dd, 8.5, 8.1)	84.1
17	3.57 (1H, s)	79.0
18a	3.65 (1H, d, 10.9)	65.0
18b	3.80 (1H, d, 10.9)	
19	6.8 (1H, s)	144.1
1-OCH$_3$	3.21 (3H, s)	57.0
6-OCH$_3$	3.50 (3H, s)	59.3
14-OCH$_3$	3.38 (3H, s)	58.1
16-OCH$_3$	3.35 (3H, s)	56.6

EXPERIMENTAL

General Experimental Procedures. 1D and 2D NMR spectra were recorded on a JEOL ECA600 spectrometer and chemical shifts were reported using residual CD$_3$OD (δ_H 3.31 and δ_C 49.0) as internal standard. HMQC experiments were optimized for $^1J_{CH}$=145 Hz and HMBC experiments for $^nJ_{CH}$=8Hz. Mass spectra were recorded on a Micromass LCT spectrometer.

Plant Material. The aerial parts of *D. sharwurense* W. T. Wang were collected in Xinjiang autonomous region of the Chinese People's Republic during flowering in July 2005. The aerial parts of this plant were identified by Prof. Shi Ming Duan of Xinjiang Institute of Ecology and Geography. A voucher specimen was deposited at the Xinjiang Technical Institute of Physics and Chemistry.

Extraction and Isolation. The aerial parts of *D. sharwurense* (2.9 kg) were dried, grounded, and extracted with 80% EtOH at rt and the EtOH extract was partitioned between $CHCl_3$ and 5% H_2SO_4 aq. Water-soluble materials, which were adjusted to pH 10 with saturated Na_2CO_3 aq., were extracted with $CHCl_3$. Water-soluble materials were extracted with BuOH. BuOH-soluble materials were subjected to a silica column ($CHCl_3$/MeOH 1:0→1:1, $CHCl_3$/MeOH/H_2O 5:5:1, and MeOH) to afford thirteen fractions. The fractions eluted by $CHCl_3$/MeOH (50:1→5:5) were subjected to an amino silica gel column ($CHCl_3$/MeOH 1:0→1:1) to afford sharwuphinine A (**1**, 11.6 mg, 0.0004%), lycoctonine (**2**, 58 mg, 0.002%),[4] and deoxymethylenelycoctonine (**3**, 145 mg, 0.005%).[5]

Sharwuphinine A (1): brown solid; $[\alpha]_D^{23}$ +25 (*c* 0.2, MeOH); IR (film) ν_{max} 3433, 2943, 2882, 2824, 1650, 1605, 1461, 1385, 1223, and 1088 cm^{-1}; UV (MeOH) λ_{max} 250 (ε 6500) nm; ^1H and ^{13}C NMR data (Table 1); ESIMS *m/z* 454 (M+H)$^+$; HRESITOFMS *m/z* 454.2446[(M+H)$^+$, calcd for $C_{23}H_{36}NO_8$, 454.2435].

Vasodilation Assay.[7] A male Wistar rat weighting 260 g was sacrificed by bleeding from carotid arteries under an anesthetization. A section of the thoracic aorta between the aortic arch and the diaphragm was removed and placed in oxygenated, modified Krebs-Henseleit solution (KHS: 118.0 mM NaCl, 4.7 mM KCl, 25.0 mM $NaHCO_3$, 1.8 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, and 11.0 mM glucose). The aorta was cleaned of loosely adhering fat and connective tissue and cut into ring preparations 3 mm in length. The tissue was placed in a well-oxygenated (95% O_2, 5% CO_2) bath of 5 mL KHS solution at 37 °C with one end connected to a tissue holder and the other to a force-displacement transducer (Nihon Kohden, TB-611T). The tissue was equilibrated for 60 min under a resting tension of 1.0 g. During this time the KHS in the tissue bath was replaced every 20 min.

After equilibration, each aortic ring was contracted by treatment with 3 x 10^{-7} M PE. The presence of functional endothelial cells was confirmed by demonstrating relaxation to 10^{-5} M acetylcholine (ACh), and aortic ring in which 80% relaxation occurred, were regarded as tissues with endothelium. When the PE-induced contraction reached a plateau, each sample (**1 – 3**, 10^{-4} M) was added.

These animal experimental studies were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University and under the supervision of the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Science, Sports Culture, and Technology of Japan.

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