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Ochrocephalamine A, a new quinolizidine alkaloid from *Oxytropis ochrocephala* Bunge

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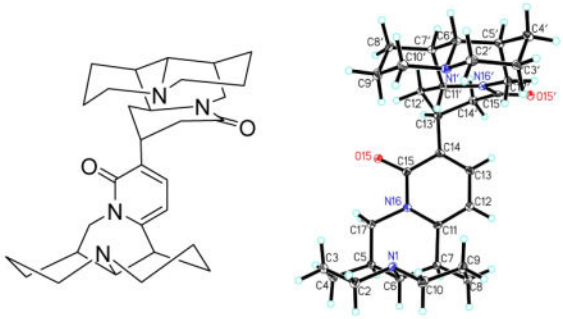
Abstract

One dimeric matrine-type alkaloid, ochrocephalamine A (**1**), was isolated from the poisonous plant *Oxytropis ochrocephala Bunge*. Its structure was elucidated by spectroscopic data and single-crystal X-ray diffraction. The insecticidal and cytotoxic activities of **1** were evaluated.

Graphical Abstract



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Supplementary Material

NMR spectra, bioactivity assay, and the X-ray crystallographic data (CIF) of **1** are available free of charge via the Internet at http://

Keywords

Poisonous plant; *Oxytropis ochrocephala Bunge*; *Oxytropis ochrocephala*; Alkaloid; Quinolizidine alkaloid

The plant *Oxytropis ochrocephala* Bunge is a common poisonous plant found in the western grasslands of China[1–2]. After ingesting the plant, livestock gradually develop a chronic neurological disease, characterized by a staggering gait and muscular incoordination, giving rise to the vivid name 'locoweed' for this poisonous plant[3]. Previous phytochemical investigations on *O. ochrocephala* provided quinolizidine and indolizidine alkaloids, as well as flavonoids and saponins[4–10].

Quinolizidine alkaloids are important natural products exhibiting a broad array of pharmacological activities, such as antineoplastic, antibacterial, antiviral, and insecticidal properties[11]. Recently, we reported the synthesis of sophoridine derivatives as potential antitumor agents[12]. As part of our ongoing research program to identify bioactive constituents, particularly from this alkaloid class, we obtained a new quinolizidine alkaloid, ochrocephalamine A (**1**), from *O. ochrocephala*. Herein, we describe the isolation, structural elucidation and bioactivity of this compound.

The whole plant (20.0 kg) of *O. ochrocephala* was percolated three times with 95% EtOH to give a crude extract (3.0 kg). The extract was concentrated to dryness under reduced pressure, followed by partitioning between CH_2Cl_2 and 2% HCl. The aqueous phase was then adjusted to pH 11 with 3% NaOH and extracted with CH_2Cl_2 to give crude alkaloids (100 g). The crude alkaloids were subjected to a silica gel column chromatography eluted with CH_2Cl_2/MeOH (1:0 to 0:1) to obtain fractions A, B and C. Fraction B was chromatographed on silica gel [CH_2Cl_2/MeOH (10:1)] and then RP-18 (30% MeOH) columns to yield ochrocephalamine A (**1**, 20 mg).

Ochrocephalamine A (**1**)[13] was obtained as a light-yellow solid (CH_2Cl_2), and its molecular formula was established as $C_{30}H_{42}N_4O_2$ by HR-EI-MS (*m/z* 490.3296 $[M]^+$, calcd 490.3308), requiring 12 degrees of unsaturation. The ^{1}H NMR data of **1** (Table 1) showed two groups of signals characteristic for a matrine-type alkaloid at δ_H 4.29 (1H, dd, J = 12.8, 4.0 Hz) and 3.11 (1H, *br* t, J = 12.8 Hz) and at 4.09 (1H, dd, J = 12.8, 7.2 Hz) and 3.80 (1H, *br* t, J = 12.8 Hz)[14]. In addition, two coupled olefinic protons [δ_H 7.30 (1H, d, J = 7.6 Hz), 6.51 (1H, d, J = 7.6 Hz)] were distinguished by NMR analysis. The ^{13}C-NMR data (Table 1) accounted for 30 carbon signals, classified as four sp^2 quaternary carbons including two lactam-CO signals at δ_C 170.9 and 164.6, two sp^2 and eight sp^3 methines, and 16 methylenes. Six sp^2 carbons accounted for 4 out of the 12 degrees of unsaturation, and the remaining 8 degrees required that **1** is an octacyclic system.

Comprehensive analysis of the 1D NMR data suggested that **1** contains two matrine-type alkaloid moieties, sophoramine and matrine[15]. Considering the octacyclic ring system of **1**, this compound was likely a dimeric alkaloid of sophoramine and matrine connected through a C-C bond. Accordingly, the methine of sophoramine at C-14 (δ_C 116.4) and methylene of matrine at C-13 (δ_C 19.2)[15] were replaced by the quaternary carbon (δ_C 130.1) and methine

(δ_C 30.0), respectively, in **1**. The HMBC correlations from H-13 to C-15, C-11 and C-13′, H_2-14′ to C-15′, C-13′ and C-14, H-13′ to C-15′ and C-14′, and H_2-12′ to C-7′, C-11′, C-13′ and C-14 confirmed the C-14–C-13′connection. Thus, the gross planar structure of ochrocephalamine A was established as shown (Figure 2a).

Determinations of the stereo configurations of **1** were particularly challenging. The ROESY correlations of H-17′α to H-5′, H-6′ to H-7′ indicated H-5′, H-6′ and H-7′ were α configuration[10]. H-11α was assigned in β-configuration on the basis of the mutual ROESY between H-17′β and H-11′[10]. The relative configuration of H-13′ could not be determined by a ROESY experiment due to unobserved correlations between H-11′ and H-13′ as well as H-11′ and H-14′. Additionally, the relative configuration of H-5, H-6 and H-7 also can't be determined by ROESY experiment due to unobserved correlations between one of them with another confirmed proton configuration. Thus, we attempted to prepare crystals of **1** for X-ray determination. Eventually, a single crystal of this compound was obtained from EtOH. The connection between C-14 and C-13′ was confirmed by X-ray crystallographic diffraction with CuKα radiation and the relative configurations of H-5, H-6, H-7 and H-13′ were α-configuration (Figure 2b)[13,16]. The absolute configurations of **1** were also determined by the refined Hooft parameter value 0.01(6) for 1892 Bijovet pairs with a probability of 1.000 as shown in Figure 2b[16].

From a biogenetic point of view, matrine could react with known sophoridine through a Michael addition reaction to give the intermediate B. Aromatization of this intermediate would then yield **1**.

We evaluated the contact toxicity of **1** against *Spodoptera litura* third-instar larvae by the micro-drip method. One μL of **1** (3 mg/mL in EtOH) was dropped on the pronotum of larvae. After five days, 50% of larvae were killed. In cytotoxicity assays, compound **1** was not active against A549, KB, KB-VIN, and MDA-MB-231 cell lines (ED_{50} > 20 μM).

O. ochrocephala is widely distributed in the grasslands of eastern and central Asia, Australia, and western North and South America, where it poses an extreme risk to grazing animals, resulting in a chronic neurological disease[1,3]. The indolizidine alkaloid swainsonine is claimed as the constituent poisonous to livestock[3]. In view of the plant itself, poisonous secondary metabolites can improve the plant's chances of survival and reproduction by deterring animals, such as sheep, cattle, and horses. In addition, quinolizidine alkaloids[8–9, 17], such as **1**, play an important role in defense against insects. Biogenetically, indolizidine and quinolizidine alkaloids originate from the same precursor L-lysine[18]. The use of one biogenetic pathway to produce two different types of alkaloids involved in plant defense against both livestock and insects, respectively, is a smart and efficient strategy to combat the survival challenge.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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13. Ochrocephalamine A (**1**): yellow crystals (EtOH); mp 159–161 °C; ESIMS m/z 491 $[M + H]^+$; HREIMS at 490.3296 $[M]^+$ (calcd. 490.3308, $C_{30}H_{42}N_4O_2$); $[\alpha]_D^{24}$ +32.2 (*c* 0.48, MeOH); UV (MeOH) λ_{max} (logε) 202 nm (4.2), 311 nm (3.9), 373 nm (2.3); IR (KBr) ν_{max} 2932, 2860, 2805, 2764, 2745, 1639, 1597, and 1554 cm^{-1}; ^1H and ^{13}C NMR data (Table 1). Crystal data for **1**: $C_{30}H_{42}N_4O_2 \cdot 6(H_2O)$, M = 598.77, monoclinic, a = 13.0528(3) Å, b =8.3014(2) Å, c = 14.8105(4) Å, α = 90.00°, β = 101.6050(10)°, γ = 90.00°, V = 1572.01(7) $Å^3$, T = 100(2) K, space group $P21$, Z = 2, μ(CuKα) = 0.745 mm^{-1}, 13217 reflections measured, 4874 independent reflections (R_{int} = 0.0386). The final R_1 values were 0.0521 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1565 ($I > 2\sigma(I)$). The final R_1 values were 0.0525 (all data). The final $wR(F^2)$ values were 0.1572 (all data). The goodness of fit on F^2 was 1.082. Flack parameter = −0.3(2). The Hooft parameter is 0.01(6) for 1892 Bijvoet pairs. Deposited to Cambridge Crystallographic Data Center with No. CCDC 1449099.
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- Ochrocephalamine A (**1**) was isolated from *Oxytropis ochrocephala Bunge.*
- The poisonous plant *O. ochrocephala* poses an extreme risk to grazing animals.
- Compound **1** is a dimeric matrine-type alkaloid.
- Its structure was solved from spectral data and single-crystal X-ray diffraction.
- Compound **1** showed contact toxicity against *Spodoptera litura* third-instar larvae.

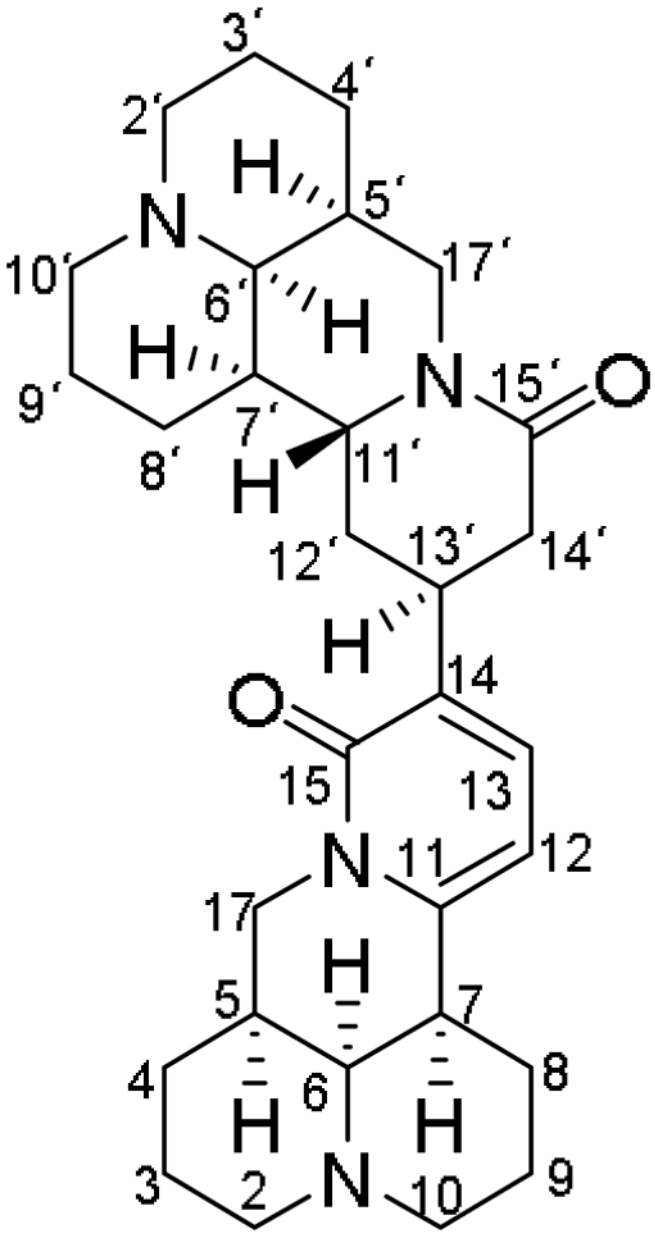


Figure 1.
The structure of ochrocephalamine A (**1**)

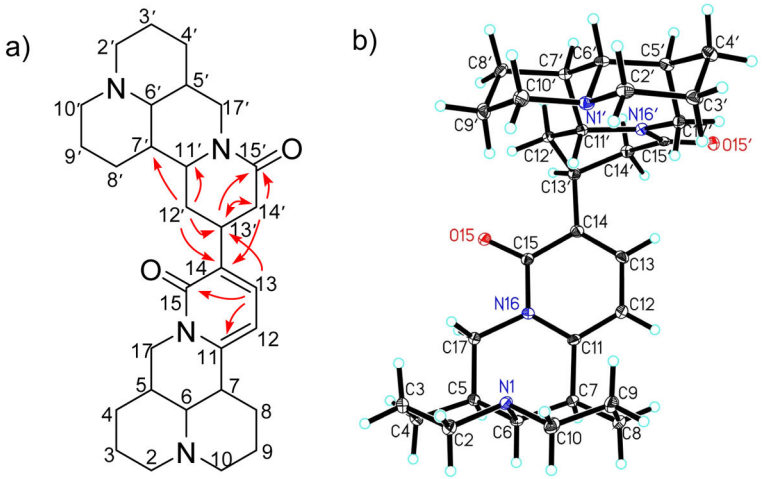
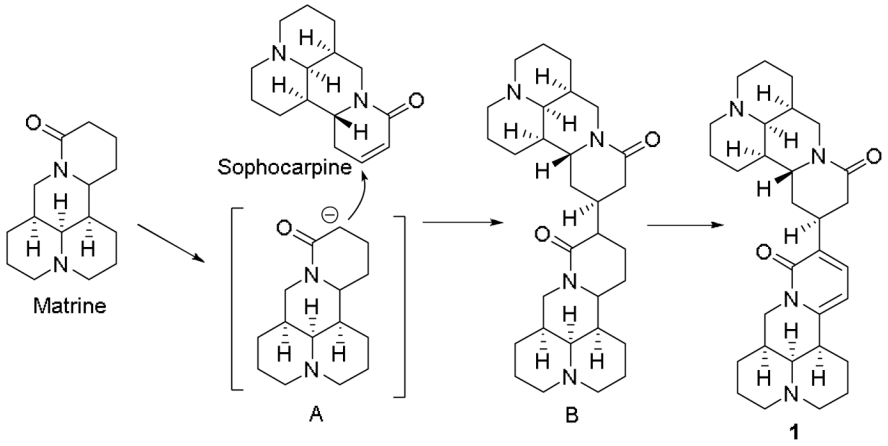


Figure 2.
a) key HMBC correlations of **1**, b) X-ray crystal structure of **1**.



Scheme 1.
Biogenetic pathway proposed of ochrocephalamine A (**1**)

Table 1

^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Data of Ochrocephalamine A (1) in CD_3OD

No	δ_H (mult, *J*)	δ_C	no.	δ_H (mult, *J*)	δ_C
2	2.82 (m)[a] 2.07 (m) [a]	57.8	2′	2.82 (m) [a] 2.07 (m) [a]	58.3
3	1.56–1.65 (m) [a]	22.2	3′	1.56–1.65 (m) [a]	21.6
4	1.79 (m) [a]	27.8	4′	1.67–1.71 (m) [a]	28.7
5	2.25 (m)	32.9	5′	1.80 (m)	37.5
6	2.33 (t, 4)	61.5	6′	2.29 (*br* s)	65.6
7	3.03 (*br* s)	39.5	7′	1.89 (m)	42.6
8	2.15 (m)	27.5	8′	2.44–2.50(m)	28.4
9	1.56–1.65 (m) [a]	22.2	9′	1.56–1.65 (m) [a]	21.3
10	2.82 (m) [a] 2.07 (m) [a]	57.6	10′	2.82 (m) [a] 2.07 (m) [a]	58.2
11		147.7	11′	3.94 (m)	53.8
12	6.51 (d, 7.6)	106.6	12′	2.14 (m)	29.8
13	7.30 (d, 7.6)	135.7	13′	3.38 (m)	30.0
14		130.1	14′	2.62(dd, 16.0, 4.0) 2.46(dd, 16.0, 8.0)	37.5
15		164.6	15′		170.9
17	4.09 (dd, 12.8, 7.2) 3.80 (*br* t, 12.8)	45.5	17′	4.29 (dd, 12.8, 4.0) 3.11 (*br* t, 12.8)	43.7

[a]overlapped.