FULL PAPER

(+)-(14*β*)-14-Ethylmatridin-15-one, a New Quinolizidine Alkaloid from the Poisonous Plant *Oxytropis ochrocephala* Bunge

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A new matrine alkaloid derivative (+)-(14*β*)-14-ethylmatridin-15-one (**1**) was isolated from the poisonous plant *Oxytropis ochrocephala* Bunge. The structure was established by spectroscopic methods, including extensive 1D- and 2D-NMR experiments.

Keywords: *Oxytropis ochrocephala*, Alkaloid, Matrine, (+)-(14*β*)-14-Ethylmatridin-15-one, Quinolizidine alkaloid.

Introduction

Matrine-type quinolizidine alkaloids represent an important class of natural products with wide range of pharmacological activities, including antineoplastic, antibacterial, and antiviral properties [1][2]. Matrine, oxymatrine, and sophoridine are three main chemical components of 'Fufang Kushen' injection, which was approved by the Chinese FDA (CFDA) in 1995 as an anticancer drug for treating non-small cell lung carcinoma, liver cancer, and gastric cancer in combination with other anticancer drugs [3].

Oxytropis ochrocephala Bunge is a common plant found on western grasslands in China that is poisonous to livestock [4 – 6]. Previous chemical investigations on *O. ochrocephala* resulted in the isolation of quinolizidine and indolizidine alkaloids, as well as flavonols and saponins [7 – 10]. In recent years, due to overgrazing, salinization, and damage from drought and rodents, this poisonous plant has spread 3 m ha across Qinghai, Ningxia, Gansu, and Xizang Provinces of China and even become the dominant species in some places [5]. However, if components, such as quinolizidine alkaloids, in this plant had a therapeutic use, harm to the grassland could be mitigated. As part of our ongoing research program on the identification of quinolizidine alkaloids, a new matrine alkaloid derivative (+)-(14*β*)-14-ethylmatridin-15-one (**1**, *Fig. 1*) was obtained from the whole plants of *O. ochrocephala*. Herein, we describe the isolation, structural elucidation, and cytotoxicity of the new compound.

Results and Discussion

Compound **1** was obtained as optically active white powder, and its molecular formula was established as $C_{17}H_{28}N_2O$ by HR-EI-MS (*m/z* 276.2209 (M^+), calc. 276.2202), requiring five degrees of unsaturation. The IR spectrum exhibited absorptions typical of lactam C=O (1637 cm^{-1}) and *trans*-quinolizidine (2796 and 2744 cm^{-1}) moieties [11]. The ^1H NMR spectrum ($CDCl_3$) of **1** (*Table*) showed one Me signal at δ(H) 0.92 (3 H, *t*, *J* = 7.6) and the characteristic signals of a matrine-type alkaloid at δ(H) 4.37 (1 H, *dd*, *J* = 12.4, 4.4 Hz, H_α–17), and 3.03 (1 H, br. *t*, *J* = 12.8 Hz, H_β–17) [12]. The ^{13}C-NMR spectra (*Table*) accounted for 17 Carbon atom signals, classified as one lactam C=O C-atom (δ(C) 172.0), ten CH_2 groups, five CH groups, and one Me group. The

[1]) These authors contributed equally to this work.

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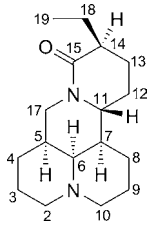

Fig. 1. Structure of **1**

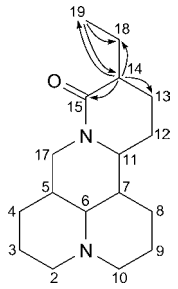

Fig. 2. Key HMBCs (H→C) of **1**

aforementioned data suggested that compound **1** and known compound matrine share a similar scaffold [13], except that the CH_2(14) C-atom at δ(C) 33.0 in the latter compound was replaced by a CH C-atom at δ(C) 43.2 in the former compound, which also contained a Me (δ(C) 11.4) and one additional CH_2 (δ(C) 24.2) not found in the latter compound. Because the molecular weight of **1** was larger than that of matrine by a C_2H_4 unit, **1** could be a 14-ethyl analog of matrine. The HMBC cross peaks from Me(19) to C(18) and C(14), from H–C(14) to C(15), C(13), C(18), and C(19) verified the location of the ethyl substitution at C(14) (*Fig. 2*).

With a *trans*-quinolizidine moiety (2796 and 2744 cm^{-1}) [1][11][12], H–C(6) must be α-oriented. The ROESY correlations from H_α–C(17) to H–C(5) and from H–C(6) to H–C(7) indicated that both H–C(5) and H–C(7) were also α-oriented. H–C(11) was assigned to a *β*-orientation on the basis of the mutual ROESY correlation between H_β–C(17) and H–C(11). Meanwhile, the ROESY correlation from H–C(11) to H–C(18) (δ(H) 1.32 – 1.42) indirectly confirmed that H–C(14) was in an α-orientation. From the above-mentioned data, the structure of **1** was unambiguously elucidated as (+)-(14*β*)-14-ethylmatridin-15-one as shown in *Figs. 2* and *3*. Compound **1** represents a new type of natural matrine derivative containing 17 C-atoms.

Compound **1** was evaluated for antiproliferative effects against A549, KB, KB-VIN, MDA-MB-231, and MCF7 cancer cells. However, it did not exhibit significant activity (IC_{50} > 20 μM). In contrast, previous studies had shown that synthetic matrine derivatives with various substituted benzylidene, benzoyl, or similar groups at the C(14) position showed potent antiproliferative effects [1]. Thus, the presence of the aliphatic ethyl group did not have the same influence on the bioactivity as the groups in the prior study.

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Experimental Part

General

Unless otherwise stated, the chemicals were acquired from commercial sources and used without further purification. All chemicals were purchased from *ACROS* (Geel, Belgium) and *Aldrich* (St.louis, MO USA). TLC: precoated silica gel GF_{254} and HF_{254} plates (*Qingdao Marine Chemical Inc.*, Qingdao, P. R. China). Column chromatography (CC): silica gel (SiO_2, 90 – 150 μm; *Qingdao Marine Chemical Inc.*). Optical rotations: *Perkin-Elmer model 241* polarimeter (Escondido, California, USA). UV Spectra: *Shimadzu UV-2401 PC* spectrometer (*Shimadzu*, Kyoto, Japan). IR Spectra: *Bio-Rad FTS-135* spectrometer (Markham, Ontario, Canada) as a KBr disk; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR spectra: *Varian Inova* (*Varian*, California, USA) (400 MHz); in $CDCl_3$; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI-MS and HR-EI-MS spectra: *VG ZABHS* and *Auto Spec-3000* spectrometers (*Waters*, Milford, USA), resp.; in *m*/*z*.

Table. ^{1}H- and ^{13}C-NMR data (400 and 100 MHz, resp.) of **1**. δ in ppm, *J* in Hz.

Position[a])	δ(H)[b])	δ(C)[b])	Position[a])	δ(H)[b])	δ(C)[b])
2	2.82 (*m*), 2.01 (*m*)	57.1	11	3.77 (*m*)	53.2
3	1.31 – 1.51 (*m*)	21.1	12	1.66 – 1.78 (*m*), 1.46 – 1.56 (*m*)	27.7
4	1.98 – 2.14 (*m*)	26.9	13	1.78 – 1.86 (*m*), 1.86 – 1.94 (*m*)	23.9
5	1.63 (*m*)	35.1	14	2.05 (*m*)	43.1
6	2.17 (*m*)	63.6	15		172.0
7	1.42 (*m*)	43.7	17	4.37 (*dd*, *J* = 12.4, 4.4), 3.03 (br. *t*, *J* = 12.8)	41.3
8	1.84 – 1.96 (*m*)	26.1	18	1.44 – 1.54 (*m*), 1.32 – 1.42 (*m*)	24.2
9	1.31 – 1.51 (*m*)	20.7	19	0.92 (*t*, *J* = 7.6)	11.4
10	2.80 (*m*), 2.01 (*m*)	57.0			

[a]) C-Atom numbering as indicated in *Fig. 1*. [b]) Measured in $CDCl_3$.

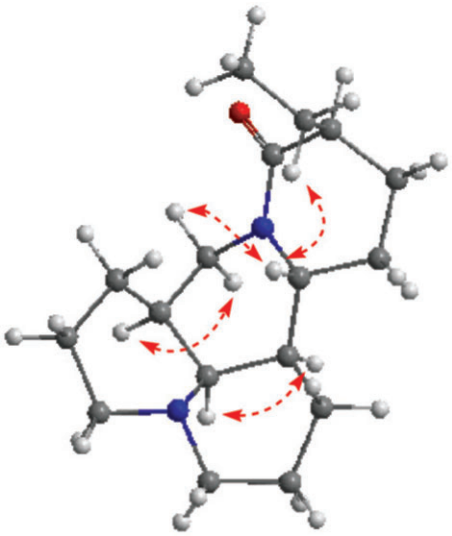

Fig. 3. ROESY (H↔H) correlations of **1**

Plant Material

The whole plants of *O. ochrocephala*, collected in Huangzhong, Qinghai Province, China, in October 2012, were identified by Prof. *Chong-Hui Mo*, Qinghai University. The voucher specimen (ZhaoBY-201203) was deposited at Northwest A&F University, Yangling, Shaanxi, China.

Extraction and Isolation

The whole plants (20.0 kg) of *O. ochrocephala* were 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 eluted with $CHCl_3$/MeOH (1:0 – 0:1) to obtain fractions **A**, **B** and **C**. The fraction **A** was chromatographed over a silica gel column ($CHCl_3$/MeOH (10:1)) followed by RP-18 (30% MeOH in water) to obtain **1**.

(+)-(14*β*)-14-Ethylmatridin-15-one (**1**). White powder. $[\alpha]_D^{20}$ = +23 (*c* = 0.18, MeOH). UV (MeOH): 205.8 (7.01). IR (KBr): 3449, 2934, 2855, 2765, 2745, 1638, 1463, 1437. ^{1}H- and ^{13}C-NMR: see *Table*. HR-EI-MS: 276.2209 (M^+, $C_{17}H_{28}N_2O^+$; calc. 276.2202).

Antiproliferative Activity Assay

Human tumor cells were cultured in 96-well plate at densities of 4000 – 11,000 cells per well in the presence of test compound. After 3 days in culture, attached cells were fixed in 10% trichloroacetic acid and then strained with 0.04% sulforhodamine B. The protein-bound dye was solubilized with 10 mM *Tris* base and absorbance at 515 nm was measured using a microplate reader operated by Gen5 software (*BioTek*, Winooski, VT, USA). The mean IC_{50} is the concentration of agent that reduces cell growth by 50% under the experimental conditions and is the average from at least three independent determinations that were reproducible and statistically significant. The following human tumor cell lines were used in the assay: lung adenocarcinoma A549, originally isolated from epidermoid carcinoma of the nasopharynx KB, multidrug-resistant KB subline KB-VIN, triple-negative breast cancer (estrogen receptor (ER)-, progesterone receptor (PgR)- and erbB2(HER2)-negative) MDA-MB-23,1 and MCF-7 (ER-positive, PgR-normal, HER2-negative). All cells were obtained from *Lineberger Comprehensive Cancer Center* (*UNC-CH*) or from *ATCC* (Rockville, MD, USA), except KB-VIN, which was a generous gift of Professor *Y.-C. Cheng*, Yale University. Cells were cultured in PRMI-1640 medium containing 25 mM HEPES, 2 mM L-glutamine (*Gibco*, St. louis, MO USA), supplemented with 10% fetal bovine serum (*Sigma*, St. louis, MO USA), 100 μg/ml streptomycin, 100 IU/ml penicillin, and 0.25 μg/ml amphotericin B (*Cellgro*, Tewksbury, MA, USA). KB-VIN stock cells were maintained in the medium containing 100 nM vincristine.

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