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Two new alkaloids from the aerial parts of *Caryopteris mongolica* Bunge.

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ABSTRACT: Two new alkaloids moncaryopterine A and moncaryopterine B were isolated from the aerial parts of *Caryopteris mongolica* Bunge. by the column chromatography and HPLC methods. Molecular structures of them were elucidated by MS, ^{1}H, ^{13}C, HSQC, HMBC, $^{1}H/^{1}H$ COSY, and $^{1}H/^{1}H$ NOESY NMR methods.

Keywords: Caryopteris mongolica Bunge. Verbenaceae, alkaloids, moncaryopterine A, moncaryopterine B

INTRODUCTION

Caryopteris mongolica Bunge. is a deciduous shrub and belongs to the Verbenaceae family. It is widely distributed throughout the Mongolian territory [1]. In addition, this plant species grows in some provinces of Hebei, Shanxi of Inner Mongolia and Gansu, China [2]. In fact, *Caryopteris mongolica* is only species grown in Mongolia, whereas about 16 other species are discovered in different places of the world.

In Mongolian traditional medicine aerial parts of *C. mongolica* have been used for the treatment of haemorrhage, chronic bronchitis and for an increasing of the muscle strength and urinary excretion [3]. In Chinese folk medicine *Caryopteris terniflora* has been used as antipyretic, expectorant and for the treatment of tuberculosis, rheumatism and cold [4]. Some species of *Caryopteris* are cultivated for a decoration arrangement and ornamental purposes.

Previous chemical investigations of *Caryopteris mongolica* showed the presence of essential oils, mono and sesquiterpenoids [5], hypolaetin-7-glucoside [6], iridiod glucosides and steriods [7, 8, 9]. Moreover, from other species of *Caryopteris* iridoids, steroid glucosides, phenylethanoids, diterpenoids, phenolic acids, α-caryopterone, a new pyranojuglone, clandonoside and its acetylated derivatives have been isolated, respectively [10, 11, 12, 13, 14]. To the best of our knowledge there are no data on alkaloids in all species of *Caryopteris.* However, we are reporting here of the molecular structure elucidation of two alkaloids isolated from the aerial parts of *C. mongolica.*

EXPERIMENTAL

Plant material. The aerial parts of *Caryopteris mongolica* Bunge. were collected from Terelj Mountain chains, vicinity of Ulaanbaatar, during the flowering period in August 2010. Dr. B. Mandakh, Institute of Botany, MAS has identified the plant species and voucher specimen was deposited at the Herbariums of the Natural Products Chemistry Laboratory, Institute of Chemistry and Chemical Technology of the Mongolian Academy of Sciences.

Extraction and isolation of alkaloids. The air dried and powdered aerial parts (3.3 kg) of *Caryopteris mongolica* were extracted with 94% ethanol at room temperature for 3 times.

The ethanol extract was evaporated to dryness and treated with 2.5% HCl, (pH=1-2). The aqueous acidic solution was extracted successively with petroleum ether and chloroform, respectively. Then the aqueous residue solution was adjusted to pH=9-10 by aq. NH_4OH (25%) and extracted with dichloromethane, which was evaporated under reduced pressure to give 1.5 g of crude total alkaloids, (0.048%). The crude total alkaloids isolated from *C. mongolica* were then carried out through sephadex LH-20. The column (150 cm × 4 cm) was eluted with chloroform-methanol (1:1) and the elutes were controlled by TLC, detected by the Dragendorff reagent and Iodine. Fractions with similar quality were combined and totally collected 6 subfractions from A to F- A-0.149 g, B-0.128 g, C-0.153 g, D-0.246 g, E-0.443 g and F-0.043 g, respectively.

The fraction D (0.246 g) was subjected to Silica gel (10 g), GF_{254}, 200-300 mesh (Qingdao Haiyang Chemical Corporation, China), the column (18 cm × 2 cm) was eluted with chloroform-methanol mixtures 50:1 to 1:1 to give 7 subfractions D_1-D_7. The fraction D_1 (77 mg) was separated on a Semipreparative HPLC using an MeOH-H_2O (10:1, v/v) solvent system at a flow rate of 3 mL/min and the peaks were detected at 208 nm. The compounds at retention times 29 and 33 min. were isolated and marked as **1**, (5 mg) and **2**, (8 mg), respectively.

Structure elucidation of alkaloids. Mass spectra **(**ESIMS) was obtained on Finnigan-LCQ^{DECA} mass spectrometer. 1D NMR: ^{1}H, (600 MHz); ^{13}C (150 MHz) and 2D NMR: HMQC, HMBC, ^{1}H/^{1}H COSY, and NOESY spectra were recorded on an Avance Bruker 600 spectrometer with TMS as an internal standard. The chemical shifts (δ) are reported in ppm and the coupling constants (J) are given in hertz, (Hz). Column chromatography (CC) was performed on self-packed open column with Sephadex LH-20 from Sweden and Silica gel $GF_{254,}$ 200-300 mesh from Qingdao Haiyang Chemical Co., Ltd. (QHCC, PR China) and Silica gel GF_{254} 0-40 mesh (QHCC) for thin layer chromatography (TLC) analyses were used, respectively. Spots on TLC plates detected under UV lamp at 254 or 365 nm and visualized by spraying with the Dragendorff reagent, pure iodine (J_2) and 8% solution of phosphomolybdic acid in ethanol. Semipreparative HPLC was equipped with a Perkin-Elmer Series 200 pump, a Perkin-Elmer Series UV/vis detector. For HPLC analysis 20μL samples were injected.

RESULTS AND DISCUSSION

The compound **1** is colorless needles, with the molecular formula $C_{18}H_{16}N_2O_2$ from ESIMS (*m/z* 315.1104 $[M + Na]^+$). The ^{1}H NMR data of the compound **1** in $CDCl_3$ (Table 1) showed that 6 aromatic protons, 4 aliphatic protons and methyl resonances at δ1.52 and at δ1.67 ppm, respectively.

Table 1. ^{1}H and ^{13}C NMR data for compounds **1** and **2** in $CDCl_3$, (δ in ppm, J in Hz)

position	δH, m, J (Hz)	δH, m, J (Hz)	δC	δC
	1	**2**	**1**	**2**
1	-	-	204.91, (CO)	205.32, (CO)
2	-	-	141.91, (C)	141.85, (C)
4	8.75, d, (1H), J=4.8	8.76, d, (1H), J=4.8	149.03, (CH)	149.00, (CH)
5	7.56, d, (1H), J=4.8	7.58, d, (1H), J=4.8	116.18, (CH)	116.38, (CH)
6	-	-	152.80, (C)	152.33, (C)
6a	-	-	44.21, (C)	43.96, (C)
7	2.54, d, (1H), J=18 2.81, d, (1H), J=18	2.49, d, (1H), J=18 2.60, d, (1H), J=18	48.97, (CH_2)	48.24, (CH_2)
8	-	-	203.70, (CO)	203.68, (CO)
9	-	-	142.20, (C)	142.11, (C)
11	8.70, d, (1H), J=4.8	8.70, d, (1H), J=4.8	148.97, (CH)	148.99, (CH)
12	7.46, d, (1H), J=4.8	7.47, d, (1H), J=4.8	116.10, (CH)	116.19, (CH)
13	-	-	150.24, (C)	150.23, (C)
14	3.41, m, (1H)	2.99, m, (1H)	36.19, (CH)	36.16, (CH)
14a	2.66, d, (1H), J=3.6	2.77, d, (1H), J=3.6	63.25, (CH)	62.19, (CH)
15	8.94, s, (1H)	8.87, s, (1H)	149.12, (CH)	149.43, (CH)
16	8.97, s, (1H)	9.02, s, (1H)	148.05, (CH)	148.08, (CH)
17	1.67, s, (3H), (-CH_3)	1.85, s, (3H), (-CH_3)	26.97, (-CH_3)	27.58, (-CH_3)
18	1.52, d, (3H), J=7.2	1.30, d, (3H), J=7.2	22.16, (-CH_3)	21.87, (-CH_3)

In addition ^{13}C NMR data revealed the presence of 18 carbons including two methyl groups and two keto groups. An analysis of the ^{1}H NMR spectrum (Table 1) in conjunction with the HSQC data confirmed that δ: two methyls at 22.16 and 26.97 ppm, a methylene protons at 48.97 ppm, eight methines at 36.19, 63.25, 116.10, 116.18, 148.05, 149.03, 149.12, and 148.97 ppm, respectively. Two keto groups δ: at 203.70, 204.91 and five quaternary carbons at 44.21, 141.91, 142.20, 150.24, 152.80 ppm, respectively, were found. HMBC cross-peaks from 2 methyl groups δ: at 1.52 ppm, d, J=7.2 Hz, 3H to C-36.19, C-63.25 and C-150.24 and 1.67 ppm, s, 3H to C-44.21, C-48.97, C-63.25 and C-152.80 ppm, respectively. Two protons of methylene δ: at 2.54, d, J=18, 1H, and 2.81, d, J=18, 1H, (CH_2) to C-26.97, C-63.25, C-152.80 and C-203.70 ppm, respectively. All interpretations of the HMBC spectrum for compound **1** showed in Table 2.

^{1}H COSY correlations of the H-4/H-5 and H-11/H-12 spin systems in the aromatic region and H-18/H-14a/H-14 coupling systems. The following HMBC correlations showed: H-18/C-14, C-13 and C-14a; H-14a/C-6a, C-7, C-14a respectively. Furthermore, the configuration of compound **1** was established by the NOESY correlations were of H-14a/H-17, H-14; H-14/H-17, H-7. Thus, the structure of the compound **1** was determined as (6aR,14R,14aS)-6a,14-dimethyl-6a,7,14,14a-tetrahydro-2,6:9,13-di(metheno)azocino[5,4-e]azecine-1,8-dione. This is naturally a new alkaloid isolated and identified for the first time from *C. mongolica* grown in the Mongolian flora, thus given a trivial name "moncaryopterine A", (**1**). The compound **2** is colorless needles, with the molecular formula $C_{18}H_{16}N_2O_2$ from ESIMS (*m/z* 315.1111 [M + Na]$^{+}$). The molecular formula and molecular weight, ^{1}H, ^{13}C NMR data of compound **2** (Table 1) were identical with the compound **1**.

Table 2. Interpretation of the HMBC spectrum for compound **1**, (δ in ppm, J in Hz

1.52, d, J=7.2, 3H, (CH_3) C-22.16,	C-36.19, (CH)	C-63.25, (CH)	C-150.24, (C)	
1.67, S, 3H, (CH_3) C-26.97	C-44.21, (C)	C-48.97, (CH_2)	C-63.25, (CH)	C-152.80, (C)
2.54, d, J=18, 1H, (CH_2) 2.81, d, J=18, 1H C-48.97	C-26.97, (CH_3) and C-44.21(C)	C-63.25, (CH)	C-152.80, (C)	C-203.70, (CO)
2.66, d, J=3.6, 1H, (CH) C-63.25	C-44.21,(C) and C-26.97, (CH_3)	C-36.19, (CH)	C-48.97, (CH_2)	C-204.91, (CO)
7.46, d, J=4.8, 1H, (CH) C-116.10	C-148.97, (C)	150.24, (C)		
7.56, d, J=4.8, 1H, (CH) C-116.18	C-152.80, (C)	149.03, (CH)		
8.70, d, J=4.8, 1H, (CH) C-148.97	C-116.10	C-142.20, (C)		
8.75, d, J=4.8, 1H, (CH) C-149.03	C-116.18	C-142.20, (C)		
8.94, S, 1H, CH C-149.12	C-142.20, (C)	C-150.24, (C)		
8.97, S, 1H, (CH) C-148.05	141.91, (C)	C-152.80, (C)		

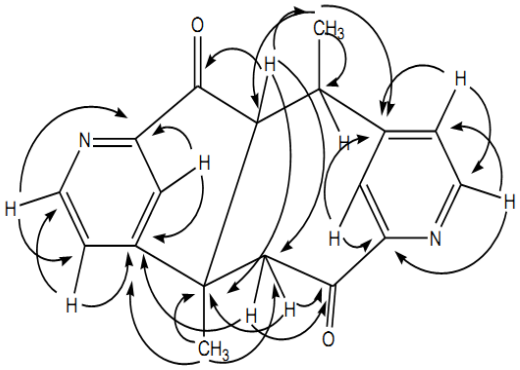


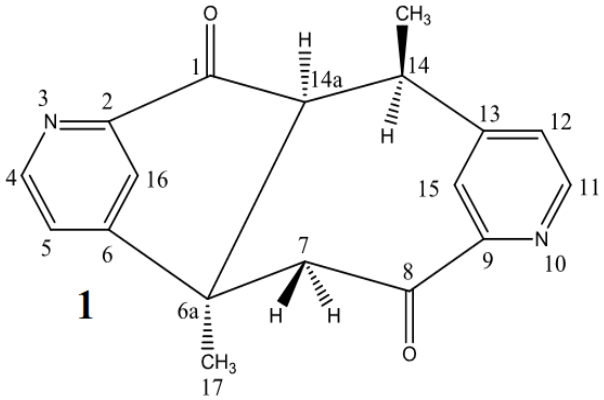
Figure 1. HMBC correlations (H→ C) for **1**

The condensation of pyridine with a cyclooktane skeleton was determined by the ^{1}H-

Compound 1. The ^{1}H NMR, (600 MHz, $CDCl_3$): see Table 1. δ8.94, (s, 1H, H-15); δ7.46, (d, 1H, J=4.8 Hz, H-12); δ7.56, (d, 1H, J=4.8 Hz, H-5); δ3.41, (m, H-14); δ2.66, (d, 1H, J=3.6 Hz, H-14a); δ8.97, (s, 1H, H-16); δ2.54, (d, 1H, J=18 Hz, H-7a); δ2.81, (d, 1H, J=18 Hz, H-7b); δ8.70, (d, 1H, J=4.8 Hz, H-11); δ8.75, (d, 1H, J=4.8Hz, H-4) and a methyl resonances at δ1.52, (d, 3H, J=7.2 Hz, CH_3 18) and δ1.67, (s, 3H, CH_3, 17). ^{13}C NMR: δ204.91 (C-1); δ141.91 (C-2); δ149.03 (C-4); δ116.18 (C-5); δ152.80 (C-6); δ44.21 (C-6a); δ48.97 (C-7); δ203.70 (C-8); δ142.20 (C-9); δ148.97 (C-11); δ116.10 (C-12); δ150.24 (C-13); δ36.19 (C-14); δ63.25 (C-14a); δ149.12 (C-15); δ148.05 (C-16); δ26.97 (C-17); δ22.16 (C-18). ESIMS *m/z* 315 $[M + Na]^+$; HRESIMS *m/z* 315.1104 $[M + Na]^+$ (calcd for $C_{18}H_{16}N_2O_2Na$, 315.32557).

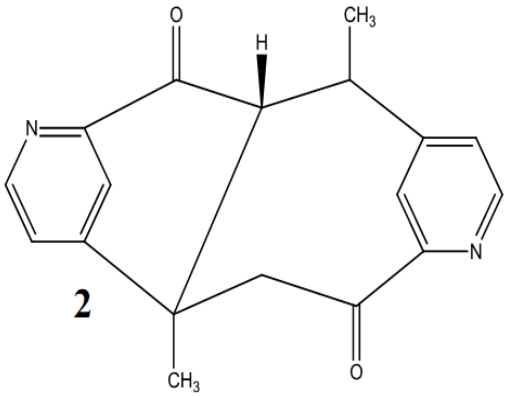
However, chemical shift of the proton 14a was observed at a weak magnetic field by $\Delta s = 0.11$ ppm. The structure of compound **2** was determined as (14aR)-6a,14-dimethyl-6a,7,14,14a-tetrahydro-2,6:9,13-di (metheno) azocino[5,4-e]azecine-1,8-dione. This is stereoisomer of the compound **1** and named "moncaryopterine B", (**2**).

Compound 2. The ^{1}H NMR (600 MHz, $CDCl_3$): see Table 1. δ8.87, (s, 1H, H-15); δ7.47, (d, 1H, J=4.8 Hz, H-4); δ2.99, (m, H-14); δ2.77, (d, 1H, J=3.6 Hz, H-14a); δ9.02, (s, 1H, H-16); δ7.58, (d, 1H, J=4.8 Hz, H-5); δ2.49, (d, 1H, J=18 Hz, H-7a); δ2.60, (d, 1H, J=18 Hz, H-7b); δ8.70, (d, 1H, J=4.8 Hz, H-11); δ8.76, (d, 1H, J=4.8Hz, H-12); and a methyl resonances at δ1.85, (s, 3H, CH_3, 17) and δ1.30, (d, 3H, J=7.2 Hz, CH_3, 18) and. ^{13}C NMR: δ205.32 (C-1); δ141.85 (C-2); δ148.08 (C-4); δ116.38 (C-5); δ152.33 (C-6); δ43.96 (C-6a); δ48.24 (C-7); δ203.68 (C-8); δ142.11 (C-9); δ148.99 (C-11); δ116.19 (C-12); δ150.23 (C-13); δ36.16 (C-14); δ62.19 (C-14a); δ149.43 (C-15); δ 149.00 (C-16); δ27.58 (C-17); δ21.87 (C-18). ESIMS *m/z* 315 $[M + Na]^+$; HRESIMS *m/z* 315.1111 $[M + Na]^+$ (calcd for $C_{18}H_{16}N_2O_2Na$), 315.32557).



1

Moncaryopterine A



2

Moncaryopterine B

CONCLUSIONS

We have isolated 2 alkaloids from the aerial parts of *Caryopteris mongolica* Bunge. grown in the Mongolian flora and molecular structures of them were elucidated by modern 1D and 2D NMR methods. These are naturally a new alkaloids isolated and identified for the first time, thus given a trivial name "moncaryopterine-A" (**1**) and "moncaryopterine-B" (**2**), respectively.

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