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Chemical constituents of aerial parts of *Thymus gobicus* and their cholinesterase inhibitory activities

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Abstract: From an acetone-water (3:2) extract of aerial parts of *Thymus gobicus* Czern. (31.1 g), compounds **1**-**8** were obtained using high-performance liquid chromatography. Based on spectroscopic data, the isolated compounds were identified as rosmarinic acid (**1**), monardic acid A (**2**), nepetoidin B (**3**), aromadendrin (**4**), apigenin (**5**), chrysoriol (**6**), apigenin 7-*O*-β-D-glucuronopyranoside (**7**), and apigenin 7-*O*-β-D-glucuronopyranoside methyl ester (**8**). Compound **2** was a (7*R*,8*R*)-diastereomer of lithospermic acid (**2a**). Although it was reported that the anti-allergic activity of lithospermic acid was higher than that of **2**, the acetylcholine inhibitory activity of **2** was higher than that of lithospermic acid.

Keywords: *Thymus gobicus, phenylpropanoid, monardic acid A, Nepetoidin B, lithospermic acid*

INTRODUCTION

Thymus gobicus belongs to the family Lamiaceae and is distributed across the Khubsugul, Khangai, Mongol Daurian, Mongolian Altai, Middle Khalkha, East Mongolia, Great Lakes Depression, Valley of the Lakes, and Gobi Altai regions of Mongolia [1]. Genus *Thymus* includes 350 species, including 9 species recorded in Mongolian territory: *T.altaicus*, *T.mongolicus*, *T.dahuricus*, *T.baicalensis*, *T.komarovii*, *T.pavlovii*, *T.roseus, T.serpyllim,* and *T.gobicus* [2]. This genus has been proven to be a rich source of essential oils; mono-, sesqui-, di-, and triterpenoids; flavonoids; phenylpropanoid oligomers; and tannins. Some of the compounds have been reported to show antibacterial [3, 4], antioxidant [5, 6], antifungal [7], and antiviral [8] activities. Essential oils are known to be important constituents characteristic of many *Thymus* plants, and about 360 volatile components have been isolated from this genus [9]. It is known that some *Thymus* plant constituents show acetylcholinesterase (AChE) inhibitory activity, and some of the essential oils are known inhibitors of this activity [1]. In Mongolian traditional medicine, *T.gobicus* is a useful and important medicinal plant. It is used for treating cold cough, whooping cough, tonsillitis, cold abdominal paints, painful menstruation, metrorrhagia, inflammation of the urinary tract, and odontalgia (toothache) [10]. Previous phytochemical studies of *T.gobicus* have been reported, in which essential oils, including thymol, cymol, terpinene, carvacrol, limonene, borneol, thymol acetate, caryophyllene, and terpineol, were identified [11].

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In addition, antioxidant [12] and some biological activities of *T.gobicus* have been studied previously [13]. In this study, three phenylpropanoids (**1**-**3**) and five flavonoids (**4**-**8**) were isolated from *T.gobicus*, and their cholinesterase inhibitory activities were estimated using human erythrocyte AChE, electric eel AChE, and horse serum butyrylcholinesterase (BChE). Monardic acid A (**2**), 7*R,* 8*R*-lithospermic acid, was also isolated from *T.gobicus*, and phenylpropanoids **2** and **3** from this plant showed AChE and BChE inhibitory activities.

EXPERIMENTAL

General: Optical rotations were measured with a Jasco P-2300 polarimeter (JASCO, Tokyo, Japan). Electronic circular dichroism (ECD) spectra were recorded on a JASCO J-700 spectropolarimeter. The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded using a Jeol JNM-AL400 FT-NMR spectrometer (JEOL, Tokyo, Japan), and the chemical shifts were reported as d values with TMS as an internal standard at 30°C (measured in CD_3OD and DMSO-d_6). The fast atom bombardment mass spectrometry (FABMS) data were obtained using a Jeol JMS700 mass spectrometer (JEOL), with a glycerol matrix. Preparative HPLC was performed using a Jasco 2089 with UV detection at 210 nm (JASCO), using the following columns: TSKgel ODS-120T (Tosoh, Tokyo, Japan, (21.5 × 300 mm), and Cosmosil 5C18-AR-II (Nacalai tesque, 20 mm x 250 mm), Mightysil RP-18 GP (Kanto Chemical, 10 mm x 250 mm).

Extraction and isolation: Dried powder of the aerial parts of *T.gobicus* (31.1 g) was extracted with acetone-water (3:2) at room temperature (two times). The extract (5.52

g) was passed through a porous polymer gel (Mitsubishi HP-20) eluted with water (**1a**, 3.60 g), MeOH-water (1:1) **(1b**, 1.14 g), and MeOH (**1c**, 1.01 g). **1c** was subjected to high-performance liquid chromatography [HPLC; column: ODS-120T, mobile phase 40% and 60% MeOH-water, 20%, 25%, and 30% CH_3CN-water containing 0.2% TFA; column AR-II, mobile phase 30% CH_3CN-water containing 0.2% TFA; column Mightysil RP-18, mobile phase 25%, 27.5%, 30% CH_3CN-water containing 0.2% TFA] to yield compounds **1** (9.3 mg), **2** (1.5 mg), **3** (1.0 mg), **4** (2.4 mg), **5** (1.2 mg), **6** (1.6 mg), **7** (3.3 mg), and **8** (2.3 mg).

Plant material: The aerial parts of *T.gobicus* were collected in Zuun modnii els, Erdene soum, Tuv province (N 47º45.349'; E 107º30.973'; H 1481m), a voucher specimen (90.21.03.09A) was deposited at the herbarium of the Laboratory of Bioorganic Chemistry and Pharmacognosy, National University of Mongolia and the herbarium of Tohoku Medical and Pharmaceutical University. The plant was identified by Dr. Ch.Sanchir, Institute of Botany, Mongolian Academy of Sciences.

Determination of cholinesterase inhibitory activities: A spectrophotometric method was used to measure the inhibition of AChE from human erythrocytes, AChE from electric eel, and BChE from horse serum (Sigma-Aldrich Co. LLC., St. Louis, USA) [14]. These assays were carried out in accordance with the procedures reported previously [15]. Samples of extracts and compounds from plants were dissolved in water or DMSO-water (1:4).

RESULTS AND DISCUSSION

Dried aerial parts of *T.gobicus* Czern. were extracted with acetone-water (3:2) then concentrated *in vacuo*. The acetone-water (3:2) extract was purified on the open column chromatography and preparative HPLCs, and eight compounds were isolated (**1**-**8**). The isolated compounds were identified based on spectroscopic data as rosmarinic acid (**1**) [16], monardic acid A (**2**) [17], nepetoidin B (**3**) [18], aromadendrin (**4**) [19], apigenin (**5**) [20], chrysoriol (**6**) [20], and apigenin 7-*O*-β-D-glucuronopyranoside (**7**) [21]. Compound **8** was isolated as the methyl ester of **7**, which might be an artifact after using MeOH. We isolated compounds **1**, **2**, **3**, **5**, **6**, and **7** previously from *Meehaniaurticifolia* [22] or *Monarda fistulosa* [17]. Rosmarinic acid (**1**) is known to be a typical phenylpropanoid dimer from Lamiaceae, Boraginaceae, Apiaceae, Rubiaceae, and Plantaginaceae [23].

Compound **1** and phenylpropanoid oligomers were named "Lamiaceae tannin", and their various biological activities, including antioxidative, anti-inflammation, antifungal, antivirus, and antimutagenic activities, have been reported previously [24]. Monardic acid A (**2**) is a phenylpropanoid trimer, that has inhibitory activities against hyaluronidase and histamine release [17]. Therefore, **1** and **2** may be related to the anti-inflammation effects of this plant. On the other hand, **2** is a diastereomer of the active phenylpropanoid trimer lithospermic acid, which is known to be a bioactive component of medicinal herbs. In the ^{1}H NMR spectrum of **2**, resonances of aliphatic protons at chiral carbons (δ 5.89, 1H, d, *J* = 5.0 Hz; 5.13, 1H, m; 4.35, 1H, dd, *J* = 5.0 Hz) were observed on the higher field than those of lithospermic acid [25]. The absolute stereochemistry of **2**, (7*R*,8*R*)-configuration, was confirmed using optical rotation data ($[\alpha]^{22}_{D}$ – 80.0). Cotton effect around 250-260 nm in the electronic circular dichroism spectra comparing with reported values [17]. In the ^{1}H NMR spectrum of **3**, the sets of *trans*-oriented (δ 7.73, 1H, d, *J* = 15.5 Hz and 6.46, 1H, d, *J* = 15.5 Hz) and *cis*-oriented (δ 7.23, 1H, d, *J* = 7.0 Hz and 5.62, 1H, d, *J* = 7.0 Hz) olefinic proton resonances and resonances of two sets of protons on the catechol moieties (δ 7.29, 1H, d, *J* = 2.0 Hz; 7.13, 1H, d, *J* = 2.0 Hz; 7.05, 1H, dd, *J* = 8.5, 2.0 Hz; 6.90, 1H, dd, *J* = 8.5, 2.0 Hz; 6.81, 1H, d, *J* = 8.5 Hz; 6.75, 1H, d, *J* = 8.5 Hz) were observed. These data indicated that **3** is nepetoidin B which is an ester of caffeic acid and phenylethanoid. It was reported that this compound showed the xanthine oxidase inhibitory activity [26]. In our screening of cholinesterase inhibitory activity using human erythrocytes AChE, the water extract of *T.gobicus* showed stronger activity than that of other plant extracts

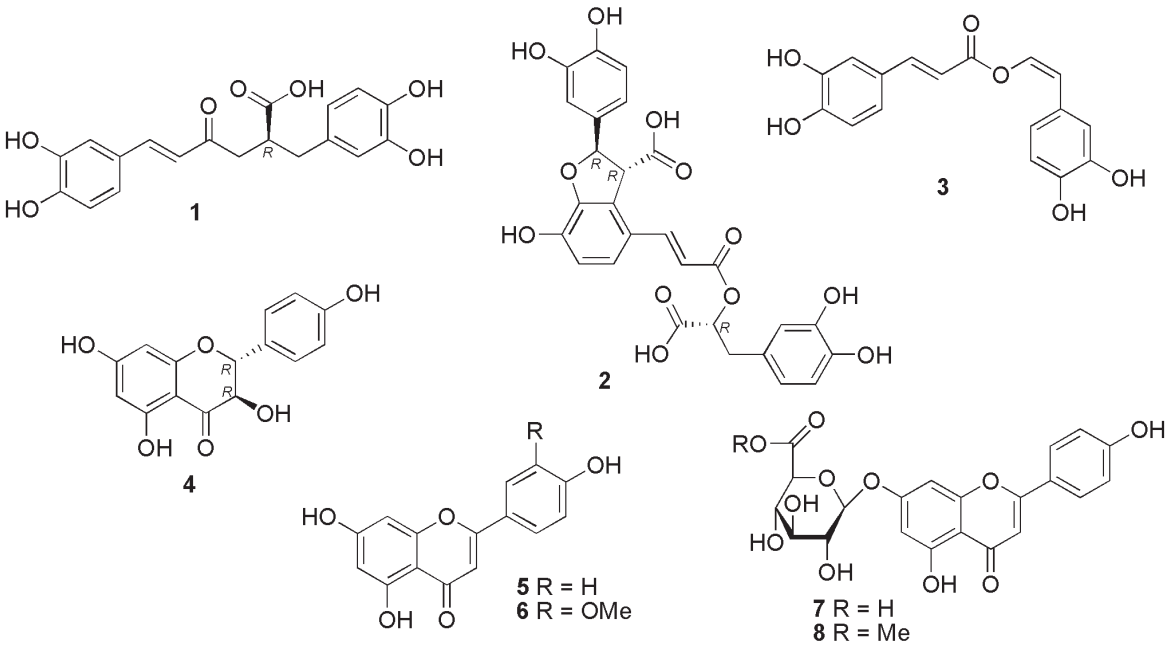

Fig. 1. Chemical structures of compounds isolated from *Thymus gobicus*

Table 1. Cholinesterase inhibitory activities of compounds from *Thymus gobicus*

Compound	AChE[(a,b,e)]	AChE[(c,e)]	BChE[(d,e)]
monardic acid A	53.1 ± 11.3	4.0 ± 29.6	> 100
nepetoidin B	37.6 ± 6.5	56.6 ± 8.2	94.7 ± 1.4
eserine[15]	0.074 ± 0.008	0.066 ± 0.014	0.18 ± 0.04
galanthamine[15]	0.62 ± 0.02	0.30 ± 0.15	84.7 ± 3.8

[a)] Compounds 1, 2a, and 3-7 were inactive (IC_{50} > 100 μM), [b)] From electric eel, [c)] From human erythrocytes, [d)] From horse serum, [e)] IC_{50} (μM) ± SEM; The treatments were replicated three times, IC_{50}, half maximal inhibitory concentration

(69.4%, 0.1 mg/ml). Then, the eight isolated compounds (**1**-**8**) and lithospermic acid (**2a**) [25] from *Lithospermum erythrorhizon* [17] were tested for their inhibitory activity against AChE from human erythrocytes, AChE from electric eel, and BChE from horse serum.

Compounds **2** and **3** both showed inhibitory activity against AChE (Table 1). On the other hand, only compound **3** had BChE inhibitory activity (Table 1). *T.gobicus* is known to contain weak cholinesterase inhibitory components as natural compounds. Caffeic acid trimers **2** and **2a** are diastereomers of each other. Compound **2a** is named lithospermic acid, which has various biological activities. In our previous report, **2a** showed stronger hyaluronidase and histamine release inhibitory activities than **2** [17]. On the other hand, only **2** showed AChE inhibitory activity. These diastereomers are difficult to distinguish from their spectroscopic data, though their biological activities are different. Compound **2a** (7*S*,8*S*-configuration) is known as an important component of medicinal plants, and **2** (7*R*,8*R*-configuration) was found in the famous medicinal herb *Thymus*. These results indicated the significance of configuration in phenylpropanoid oligomers.

Monardic acid A (**2**): Pale blown amorphous solid; $[\alpha]^{22}_{D}$ – 80.0 (c 0.08, MeOH); ECD (*c* 0.00008, MeOH) nm ([θ]) 228 (23200), 241 (14200), 252 (20400), 287 (+8900) nm; ^{1}H NMR: (CD_3OD, 400 MHz), δ: 7.81 (1H, d, *J* = 16.0 Hz), 7.21 (1H, d, *J* = 8.5 Hz), 6.81 (1H, d, *J* = 8.5 Hz), 6.80 (1H, d, *J* = 2.0 Hz), 6.77 (1H, d, *J* = 8.5 Hz), 6.76 (1H, d, *J* = 2.0 Hz), 6.73 (1H, dd, *J* = 8.5, 2.0 Hz), 6.68 (1H, d, *J* = 8.0 Hz), 6.62 (1H, dd, *J* = 8.0, 2.0 Hz), 6.34 (1H, d, *J* = 16.0 Hz), 5.89 (1H, d, *J* = 5.0 Hz), 5.13 (1H, m), 4.35 (1H, dd, *J* = 5.0 Hz), 3.07 (1H, dd, *J* = 14.0, 4.5 Hz), 2.97 (1H, dd, *J* = 14.0, 8.0 Hz). FABMS (positive) *m/z* 539 $[M+H]^+$ ($C_{27}H_{23}O_{12}$), 561 $[M+Na]^+$ ($C_{27}H_{22}O_{12}Na$).

Nepetoidin B (**3**): Pale blown amorphous solid; ^{1}H NMR: (CD_3OD, 400 MHz), δ: 7.73 (1H, d, *J* = 15.5 Hz), 7.29 (1H,d, *J* = 2.0 Hz), 7.23 (1H, d, *J* = 7.0 Hz), 7.13 (1H, d, *J* = 2.0 Hz), 7.05 (1H, dd, *J* = 8.5, 2.0 Hz), 6.90 (1H, dd, *J* = 8.5, 2.0 Hz), 6.81 (1H, d, *J* = 8.5 Hz), 6.75 (1H, d, *J* = 8.5 Hz), 6.46 (1H, d, *J* = 15.5 Hz), 5.62 (1H, d, *J* = 7.0 Hz). FABMS (positive) *m/z* 315 $[M+H]^+$ ($C_{17}H_{15}O_6$), 337 $[M+Na]^+$ ($C_{17}H_{14}O_6Na$).

CONCLUSIONS

Rosmarinic acid, monardic acid A, nepetoidin B, aromadendrin, apigenin, chrysoriol, apigenin 7-*O*-β-D-glucuronopyranoside, and apigenin 7-*O*-β-D-glucuronopyranoside methyl ester compounds were obtained from the aerial parts of *Thymus gobicus*. Acetylcholinesterase and butyrylcholinesterase inhibitory activities of isolated compounds were evaluated, monardic acid A and nepetoidin B showed moderate activities. Monardic acid A is a (7*R*, 8*R*)-diastereomer of lithospermic acid. The acetylcholine inhibitory activity of monardic acid A was higher than that of lithospermic acid.

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