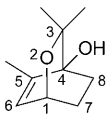
Two New Monoterpenes from *Mentha haplocalyx* BRIQ.

by **Gaimei She**, **Chao Xu**, **Bin Liu***, and **Renbing Shi**

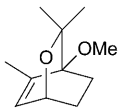
School of Chinese Pharmacy, Beijing University of Chinese Medicine, Beijing 100102, P. R. China (phone: +86-10-84738629; fax: +86-10-84738611; e-mail: liubinyn67@163.com)

Two new monoterpenes with a skeleton similar to the 1,8-cineole skeleton, *i.e.*, (1*R*,4*R*)-3,3,5-trimethyl-2-oxabicyclo[2.2.2]oct-5-en-4-ol (**1**) and (1*R*,4*R*)-4-methoxy-3,3,5-trimethyl-2-oxabicyclo-[2.2.2]oct-5-ene (**2**), were isolated from an aqueous acetone extract of the aerial parts of *Mentha haplocalyx*, together with three known compounds. Their structures were elucidated by extensive spectroscopic methods and by comparison with reference values.

Introduction. – *Mentha haplocalyx* is widely distributed in the Jiangsu, Anhui, Jiangxi, and Zhejiang Provinces of China. It is not only used as popular vegetable but also widely used for the treatment of nerve-center, breath, procreation, and digestive affections in China [1]. It is notable that *M. haplocalyx* is the most important source of essential-oil production in China [2]. Several flavonoids have been isolated from *M. haplocalyx* [3]. As a part of our phytochemical investigation on medicinal plants and to discover new bioactive natural products [4–8], we report the isolation and structural determination of two new monoterpenoids, (1*R*,4*R*)-3,3,5-trimethyl-2-oxabicyclo-[2.2.2]oct-5-en-4-ol (**1**) and (1*R*,4*R*)-4-methoxy-3,3,5-trimethyl-2-oxabicyclo[2.2.2]oct-5-ene (**2**), together with three known monoterpenoid glucosides, (1*R*,2*R*,4*S*)-*trans*-1,8-cineol-2-ol 2-(*β*-D-glucopyranoside) [9], petroside [10] and (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigm-7-ene-3,5,6,9-tetrol 3-(*β*-D-glucopyranoside) [11], from a 70% aqueous acetone extract of *M. haplocalyx*. Their structures were elucidated on the basis of spectroscopic methods, including 2D-NMR (HMBC, HMQC, ^{1}H,^{1}H-COSY, and NOESY), and by comparison with reference values.



1



2

Results and Discussion. – The chlorophyll-removal fraction from a 70% aqueous acetone extract of the aerial parts of *M. haplocalyx* was purified by repeated column chromatography to afford the two novel monoterpenoids **1** and **2** and three known monoterpenoid glucosides.

Compound **1** was obtained as colorless needles and gave a dark green color with H_2SO_4 spray reagent. The molecular formula was assigned as $C_{10}H_{16}O_2$ on the basis of the HR-EI-MS (*m/z* 168.1152 (M^+)) and in combination with the presence of ten C-atom signals in the ^{13}C-NMR spectrum. The ^{1}H-NMR signals of three Me groups at δ(H) 1.15 (3 H) and 1.28 (6 H), together with those of two CH_2 and two CH groups at δ(H) 2.12–5.59 including an olefinic H-atom, supported a bicyclic structure for **1**. The ^{1}H-NMR chemical shifts of **1** were similar to those of reported 1,8-cineolols [12–17] (1,8-cinneole = 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane), showing the signals of three Me groups (δ(H) 1.15 (*s*, Me_{endo}–C(3)), 1.28 (*s*, Me–C(5)), and 1.28 (*s*, Me_{oxo}–C(3))), two CH_2 groups (δ(H) 2.12 (*td*, H_a–C(7)), 2.22 (*td*, H_b–C(7)), 1.70–1.60 (*m*, CH_2(8))), and a CH group (δ(H) 3.92 (*d*, J = 1.9 Hz, H–C(1)). In addition, the presence of an olefinic H-atom (δ(H) 5.59 (*dd*, J = 2.9, 2.3 Hz, H–C(6))) was observed, suggesting that **1** has an olefinic bond. This was confirmed by the ^{13}C-NMR and DEPT spectra showing the presence of three Me, two CH_2, and two CH groups including one olefinic C-atom and one C-atom bearing an OH group, together with three quaternary C-atoms. In accordance with *Bredt*'s rule, the C=C bond can only be between C(5) and C(6) in the bicyclic structure of **1**. Moreover, the ^{1}H,^{1}H-COSY cross-peaks H–C(6)/H–C(1) and H–C(1)/CH_2(7)/CH_2(8) indicated the presence of a C=CHCH-OCH_2CH_2 fragment in **1**. This suggested that the Me–C(1) of a 1,8-cineole was linked to C(5) in **1**, *i.e.*, to the olefinic quaternary C-atom **1**, and the OH group was attached to C(4). This was further confirmed by the HMBC experiment, in which correlations of the Me groups at δ(H) 1.28 with the olefinic C-atoms at δ(C) 147.0 and 122.3 and C(4) at δ(C) 72.9 were observed, respectively. Furthermore, the HMBC spectrum of **1** revealed that H–C(1) (δ(H) 3.92) and H–C(6) (δ(H) 5.59) were coupled to C(3) (δ(C) 72.4), C(6) (δ(C) 122.3), and C(5) (δ(C) 147.0), together with C(7) (δ(C) 24.2), C(4) (δ(C) 72.9), and C(5) (δ(C) 147.0), respectively. Other HMBC correlations confirmed the structure of **1**. Thus, the 2D-NMR data allowed to deduce compound **1** as 3,3,5-trimethyl-2-oxabicyclo[2.2.2]oct-5-en-4-ol.

The molecular formula of the CH_2-homologous compound **2** was determined to be $C_{11}H_{18}O_2$ based on the negative HR-EI-MS (*m/z* 182.1290 (M^+)). The ^{1}H- and ^{13}C-NMR spectroscopic feature resembled those of **1**, suggesting similar structures for both compounds. The only difference was the presence of a MeO group in **2**. The typical ^{1}H- and ^{13}C-NMR signals at δ(H) 3.05 (*s*) and δ(C) 50.5 indicated the presence of a MeO group in **2** instead of the OH group of **1**. Therefore, the structure of **2** was determined to be 4-methoxy-3,3,5-trimethyl-2-oxabicyclo[2.2.2]oct-5-ene.

The coupling constants of 1.9 Hz for H–C(1)/H–C(6), 2.9 Hz for H–C(6)/H_{anti}–C(7) (long-rang coupling), and 2.3 Hz for H–C(6)/H_{anti}–C(8) (long-rang coupling) for **1** and **2** were in good agreement with those of (1*S*,4*R*,6*R*)-1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-6-ol [18–22], therefore **1** and **2** must have *rel*-(1*R*,4*R*)-configuration. This is in accord with the most stable structure of compounds **1** and **2** found by the systematic-search method in the Sybyl 6.6 program. Then, compounds **1** and **2** are levorotary and have identical absolute configurations.

This work was supported by the *Research Fund for the Doctoral Program of Higher Education of China* (No. 20100013110002) and the *Research Fund for Self-selected Topic of Beijing University of Chinese Medicine* (No. 0100600122).

Experimental Part

General. Column chromatography (CC): *Dianion HP 2MGL*, silica gel (SiO_2), *MCI* gel *CHP 20P* and *ODS-A*. TLC: SiO_2 *G* plates, eluent $CHCl_3$/MeOH/H_2O 8:2:0.2 or 7:3:0.5. Optical rotations: *PE-343* polarimeter. IR Spectra: *IR-450* spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR, HSQC, HMBC, $^1H,^1H$-COSY, and ROESY Data: *Bruker-AM-400* and *-DRX-500* spectrometers; at 500 and 400 MHz (^1H) and 125 and 100 MHz (^{13}C); in CD_3OD; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI- and HR-EI-MS: *Apex-II-FT-ICR* and *VG-ZAB-HS* spectrometer; in *m*/*z* (rel. %).

Plant Material. The aerial parts of *M. haplocalyx* were purchased from *Beijing TongRenTang Medicinal Material Co.*, Beijing, China, in June 2006, and identified by *B. L.*, Beijing University of Chinese Medicine.

Extraction and Isolation. The aerial parts of *M. haplocalyx* BRIQ. (5.0 kg) were extracted 3 × with 70% aq. acetone (3 × 10 l) at r.t. After evaporation of the org. solvent, the aq. soln. was extracted with Et_2O to yield an Et_2O and aq. fraction. The aq. fraction was concentrated to a small volume (200 ml) and subjected to CC (*Dianion HP 2MGL*, H_2O/MeOH 1:0 → 0:1): *Fractions 1–6*. *Fr. 2* (10 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 9:1 → 7:3): *(3S,5R,6R,7E,9S)-megastigm-7-ene-3,5,6,9-tetrol 3-(β-D-glucopyranoside)* (13 mg) and *(1R,2R,4S)*-trans-*1,8-cineol-2-ol-2-(β-D-glucopyranoside)* (15 mg). *Fr. 3* (36 g) was subjected to CC (*MCI* gel *CHP20P* and *ODS-A*; H_2O/MeOH 1:0 → 0:1): **1** (65 mg), **2** (10 mg), and *petroside* (8 mg).

(1R,4R)-3,3,5-Trimethyl-2-oxabicyclo[2.2.2]oct-5-en-4-ol (**1**): Colorless needles. $[\alpha]_D^{20} = -12.6$ ($c = 0.005$, MeOH). IR (KBr): 3381, 3296, 2975, 1148, 1119, 1072, 1030. ^1H-NMR (500 MHz): 1.15 (*s*, Me_{endo}–C(3)); 1.29 (*s*, Me_{exo}–C(3), Me–C(5)); 1.60–1.70 (*m*, CH_2(8)); 2.12 (*ddd*, *J* = 5.8, 8.0, 13.0, H_a–C(7)); 2.22 (*ddd*, *J* = 5.8, 11.5, 12.0, H_b–C(7)); 3.92 (*d*, *J* = 1.9, H–C(1)); 5.59 (*dd*, *J* = 2.9, 2.3, H–C(6)). ^{13}C-NMR (125 MHz): 147.0 (*s*, C(5)); 122.3 (*d*, C(6)); 74.7 (*d*, C(1)); 72.9 (*s*, C(4)); 72.5 (*s*, C(3)); 34.7 (*t*, C(8)); 29.0 (*q*, Me_{endo}–C(3), *Me*–C(5)); 24.1 (*d*, C(7)); 21.8 (*q*, Me_{exo}–C(3)). EI-MS: 168 (M^+), 153 ($[M-CH_3]^+$), 150 ($[M-H_2O]^+$), 135, 122, 110, 107, 95, 70, 59. HR-EI-MS: 168.1152 (M^+, $C_{10}H_{16}O_2^+$; calc. 168.1150).

(1R,4R)-4-Methoxy-3,3,5-trimethyl-2-oxabicyclo[2.2.2]oct-5-ene (**2**): Colorless needles. $[\alpha]_D^{20} = -21.6$ ($c = 0.002$, MeOH). ^1H-NMR (500 MHz): 1.15 (*s*, Me_{endo}–C(3)); 1.27 (*s*, Me_{exo}–C(3), Me–C(5)); 1.63–170 (*m*, CH_2(8)); 2.03 (*ddd*, *J* = 5.6, 8.0, 13.0, H_a–C(7)); 2.12 (*ddd*, *J* = 5.6, 11.5, 12.0, H_b–C(7)); 3.05 (*s*, MeO–C(4)); 3.93 (*d*, *J* = 1.9, H–C(1)); 5.55 (*dd*, *J* = 4.4, 2.3, H–C(6)). ^{13}C-NMR (125 MHz): 143.4 (*s*, C(5)); 126.5 (*d*, C(6)); 78.1 (*s*, C(4)); 74.6 (*d*, C(1)); 72.4 (*s*, C(3)); 50.5 (*q*, MeO); 34.5 (*t*, C(8)); 25.8 (*q*, *Me*–C(5)); 25.6 (*q*, Me_{endo}–C(3)); 23.5 (*d*, C(7)); 22.0 (*s*, Me_{exo}–C(3)). EI-MS: 182 (M^+), 168 ($[M-14]^+$), 150, 149, 121, 110, 107, 95, 73. HR-EI-MS: 182.1290 (M^+, $C_{11}H_{18}O_2^+$; calc. 182.1307).

REFERENCES

[1] C. Y. Liang, W. L. Li, H. Q. Zhang, B. R. Ren, *Chin. Wild Plant Res.* **2003**, *22*, 9.
[2] X. G. Wei, Y. Dong, Q. X. Cui, G. L. Zhang, *Yantai Norm. Univ. J.* **2003**, *19*, 116.
[3] Y. Liu, Y. H. Zhang, R. B. Shi, *China J. Chin. Mater. Med.* **2005**, *30*, 1086.
[4] K.-J. Wang, Y.-J. Zhang, C.-R. Yang, *Chem. Biodiversity* **2006**, *3*, 1317.
[5] K.-J. Wang, C.-R. Yang, Y.-J. Zhang, *Food Chem.* **2006**, *101*, 365.
[6] G. M. She, Z. Q. Guo, H. N. Lü, D. M. She, *Molecules* **2009**, *14*, 4190.
[7] G.-M. She, D. Wang, S.-F. Zeng, C.-R. Yang, Y.-J. Zhang, *J. Food Sci.* **2008**, *73*, 476.
[8] G.-M. She, Y.-J. Zhang, C.-R. Yang, *Chem. Biodiversity* **2009**, *6*, 875.
[9] Y. Z. Wang, W. S. Feng, R. B. Shi, B. Liu, Y. Y. Jiang, *Chin. Pharm. J.* **2008**, *43*, 669.
[10] M. Yoshikawa, T. Uemura, H. Shimoda, A. Kishi, Y. Kawahara, H. Matsuda, *Chem. Pharm. Bull.* **2000**, *48*, 1039.
[11] T. Hasegawa, F. Takano, T. Takata, M. Niiyama, T. Ohta, *Phytochemistry* **2008**, *69*, 747.
[12] H. Nishimura, Y. Noma, J. Mizutani, *Agric. Biol. Chem.* **1982**, *46*, 2601.
[13] K. Kubota, K. Nakamura, A. Kobayashi, *J. Agric. Food Chem.* **1998**, *46*, 5244.
[14] Y. Sekiwa, Y. Mizuno, Y. Tamamoto, K. Kubota, A. Kobayashi, H. Koshino, *Biosci. Biotechnol. Biochem.* **1999**, *63*, 384.
[15] Y. Someya, A. Kobayashi, K. Kubota, *Biosci. Biotechnol. Biochem.* **2001**, *65*, 950.

[16] T. M. Flynn, I. A. Southwell, *Aust. J. Chem.* **1979**, *32*, 2093.
[17] R. M. Carman, K. D. Klika, *Aust. J. Chem.* **1992**, *45*, 651.
[18] I. C. Macrae, V. Alberts, R. M. Carman, I. M. Shaw, *Aust. J. Chem.* **1979**, *32*, 917.
[19] M. Miyazawa, H. Kameoka, K. Morinaga, K. Negoro, N. Mura, *J. Agric. Food Chem.* **1989**, *37*, 222.
[20] S. M. Bitteur, R. L. Baumes, C. L. Bayonove, G. Versini, C. A. Martin, A. D. Serra, *J. Agric. Food Chem.* **1990**, *38*, 1210.
[21] K. Kubota, Y. Someya, R. Yoshida, A. Kobayashi, T.-i. Morita, H. Koshino, *J. Agric. Food Chem.* **1999**, *47*, 685.
[22] M. Miyazawa, Y. Hashimoto, *J. Agric. Food Chem.* **2002**, *50*, 3522.

Received April 13, 2010