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Highly Oxygenated Monoterpenes from *Eupatorium fortunei*

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Abstract: A pair of epimers of highly-oxygenated monoterpenes were isolated from the traditional Chinese medicine *Eupatorium fortunei*. Their structures were elucidated on the basis of the spectral analysis as (1R*, 2S*, 3R*, 4R*, 6S*)-1, 2, 3, 6-tetrehydroxy-*p*-menthane (**1**) and (1S*, 2S*, 3S*, 4R*, 6R*)-1, 2, 3, 6-tetrehydroxy-*p*-menthane (**2**).

Keywords: *Eupatorium fortunei*, traditional Chinese medicine, epimer, highly-oxygenated monoterpenes.

Eupatorium fortunei is a traditional Chinese medicine, which is widespread in China and used for the treatment of dropsical swelling, chills and fever, as a diuretic, antipyretic[1]. A Japanese research group has found a series of thymol derivatives from *Eupatorium fortunei*[2]. In order to search for its biologically active constituents our group carried out a further phytochemical study on this plant and two new highly-oxygenated monoterpenes were isolated from the EtOAc part of its MeOH extract. The EtOAc part showed weak cytotoxicity: IC_{50}=69.5 and 86.1 μg/mL against HL-60 and SMMC-7721 cells by SRB method.

Compound **1** was obtained as colorless oil, $[\alpha]_D^{14}$ +4 (*c* 0.15, CH_2Cl_2). Its IR spectrum showed obvious absorption band for hydroxy groups at 3423 cm^{-1}. The HRSIMS showed $[M+H]^+$ at *m/z* 205.0536 (calcd. for $C_{10}H_{20}O_4$+H^+ 205.1434) and the EIMS gave a molecular ion peak at *m/z* 204 and fragment ion peaks at *m/z* 186 $[M-H_2O]^+$, 168 $[M-2H_2O]^+$, 153 $[M-2H_2O-Me]^+$, 125 $[M-2H_2O$-isopropyl$]^+$ and 107 $[M-3H_2O$-isopropyl$]^+$, corresponding to a molecular formula $C_{10}H_{20}O_4$. The ^{1}H-NMR, ^{13}C-NMR and DEPT spectra (**Table 1**) showed signals for a quaternary carbon, five methines, a methylene and three methyls. The proton signals of three methyl groups were at δ_H 1.39 (s, 3H), 0.92 (d, 3H, *J* = 7.2 Hz), 0.78 (d, 3H, *J* = 7.2 Hz) and three of five methine protons were bearing oxygen signals at δ_H 3.69 (d, 1H, *J* =9.3 Hz), 3.96 (dd, 1H, *J* = 9.3, 11.4 Hz) and 3.77 (t, 1H, *J* = 2.4 Hz) in the ^{1}H-NMR spectrum. The chemical shift of the quaternary carbon was at δ_C 74.6 in the ^{13}C-NMR spectrum. Thus compound **1** was deduced as a menthane monoterpene derivative with four hydroxys[3]. The cross-peaks of H-3 (δ 3.96) with H-2 (δ 3.69) and H-4 (δ 1.97); H-4 with H-3, H-5 (δ 1.59, 1.77) and

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H-8 (δ 2.30); H-6 (δ 3.77) with H-5 in the ^{1}H-^{1}H COSY of **1** and the correlations of a methyl singlet at δ_H 1.39 with C-1 (δ 74.6), C-2 (δ 76.7) and C-6 (δ 72.9) as well as other correlations in the HMBC (**Figure 2**) showed that the four hydroxys were located at C-1, C-2, C-3 and C-6, respectively and the isopropyl group was present on C-4, indicated that compound **1** was 1, 2, 3, 6-tetrahydroxy-*p*-menthane. The relative stereo-structure of **1** could be determined by the coupling pattern of cyclic protons in its ^{1}H-NMR spectrum. The large coupling constants of H-3 with H-2 and H-4 ($J_{3,2}$ = 9.3 Hz, $J_{3,4}$ = 11.4 Hz) showed that C_2-H, C_3-H and C_4-H were axial protons, and the small coupling constants of H-6 with H-5_{ax} and H-5_{eq} ($J_{6,5a}$ = $J_{6,5e}$ = 2.4 Hz) was characteristic of the equatorial H-6 (**Figure 1**). The configuration of C_1 was determined from NOE experiments: irradiation of the methyl group at δ 1.39 (H-7) enhanced the signal at δ 3.69 (H-2). Therefore the structure of **1** was identified as (1R*, 2S*, 3R*, 4R*, 6S*)-1, 2, 3, 6-tetrehydroxy-*p*-menthane.

Compound **2** was obtained as colorless oil, $[\alpha]_D^{17}$ -36 (*c* 1.4, CH_2Cl_2). Its IR and MS were very similar to these of **1**. The molecular formula was the same as that of compound **1** ($C_{10}H_{20}O_4$). Its HRSIMS due to $[M+H]^+$ was at *m/z* 205.0521 (calcd. for $[C_{10}H_{20}O_4+H]^+$ 205.1434) and EIMS due to $[M]^+$ was at *m/z* 204 and fragment ion peaks at *m/z* 189 $[M-Me]^+$, 186 $[M-H_2O]^+$, 161 [M-isopropyl]$^+$, 143 $[M-H_2O$-isopropyl$]^+$ and 125 $[M-2H_2O$-isopropyl$]^+$. **2** and **1** had similar chemical shifts of protons but obvious different coupling constants in ^{1}H-NMR (**Table 1**), and their shifts of carbons had small difference in ^{13}C-NMR (**Table 1**), which indicated that compound **2** was a stereo-isomer of **1**. The protons and carbons were easily assigned by HMBC (**Figure 2**) and ^{1}H-^{1}H COSY experiments. Stereochemically, the coupling constants of H-4 with H-5_{ax} ($J_{4,5a}$ = 13.5 Hz), H-6 with H-5_{ax} ($J_{6,5a}$ = 10.8 Hz) and H-3 with H-2 and H-4 ($J_{3,2}$ =$J_{3,4}$ = 2.7 Hz) showed that C_4-H and C_6-H were axial protons and C_3-H was equatorial proton. In the NOE experiment, the enhancement between H-6 and H-2 could be observed but no enhancement between the methyl at δ 1.45 (H-7) and H-6 or H-2, indicating that the methyl (H-7) was at opposite side of C_2-H and C_6-H. Thus compound **2** was deduced to be (1S*, 2S*, 3S*, 4R*, 6R*)-1, 2, 3, 6-tetrahydroxy-*p*-menthane.

Table 1 ^{1}H-NMR (300 MHz), ^{13}C-NMR (100 MHz) and DEPT data of compounds **1** and **2** ($CDCl_3$, δ ppm, J Hz)

No.	**1** δ_H	**2** δ_H	No.	**1** δ_C	**2** δ_C	DEPT
1			1	74.6	74.3	C
2	3.69 d (9.3)	3.81 d (2.7)	2	76.7	77.8	CH
3	3.96 dd (9.3, 11.4)	4.15 t (2.7)	3	68.9	70.4	CH
4	1.97 ddt (11.7, 11.4, 3)	2.06 dt (13.5, 2.7)	4	41.4	43.5	CH
5a	1.77 m	1.70 m	5	26.8	32.7	CH_2
5e	1.59 m	1.78 m				
6	3.77 t (2.4)	4.29 dd (10.8, 2.4)	6	72.9	66.9	CH
7	1.39 s	1.45 s	7	23.9	20.6	CH_3
8	2.30 m	1.68 m	8	27.8	27.8	CH
9	0.92 d (7.2)	0.97 d (6.6)	9	20.9	20.9	CH_3
10	0.78 d (7.2)	0.97 d (6.6)	10	14.9	20.9	CH_3

Figure 1 Structures of compounds **1** and **2**

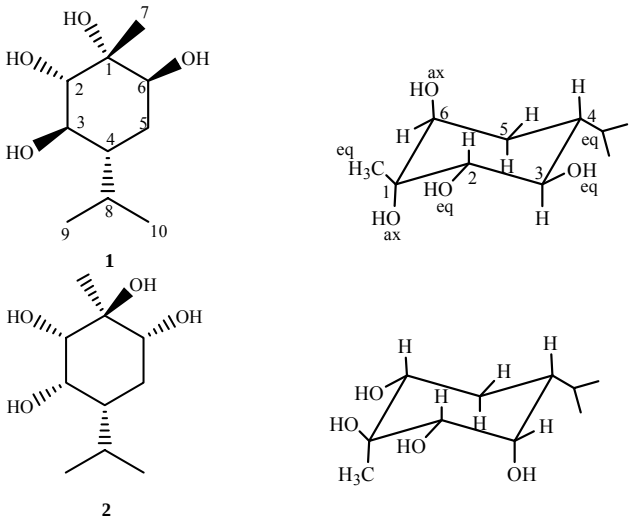
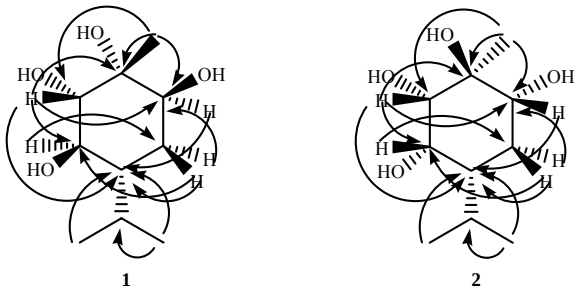


Figure 2 Correlation of HMBC of compounds **1** and **2**



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