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MONOTERPENES AND OTHER CHEMICAL CONSTITUENTS FROM THE AERIAL PARTS OF *Inula japonica*

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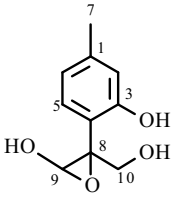
Inula japonica Thunb., as a well-known traditional herbal medicine, is widely distributed in China, Japan, and Korea [1]. Modern pharmacological study has also shown its diverse biological activities, such as antitumor [1–3], antidiabetic [4], and hypolipidemic [4] activities. In order to reveal the effective constituents of this plant, a continuous research was carried out in recent years. Our previous investigation on the aerial parts of *I. japonica* have led to the isolation of anthranilic acid derivatives [2], sesquiterpenes [1], and diterpenes [5]. In this paper, we described the isolation and structure elucidation of several monoterpenes, including one new thymol derivative and other chemical constituents.

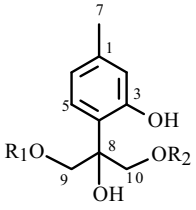
The aerial parts of *I. japonica* were collected in Anhui province, P. R. China, in October, 2006, and were authenticated by Prof. Huang Bao-Kang, Department of Pharmacognosy, School of Pharmacy, Second Military Medical University. A voucher specimen (No. 2007XFH1) was deposited at the School of Pharmacy, Shanghai Jiao Tong University.

The dried aerial parts of *I. japonica* (20.0 kg) were powdered and extracted with 95% ethanol for three times at room temperature. The ethanolic extract was successively partitioned with petroleum ether (PE), CH_2Cl_2, EtOAc, and *n*-BuOH, respectively. The EtOAc fraction (30.1 g) was chromatographed on a silica gel column eluting with a step gradient of CH_2Cl_2–MeOH (100:0, 50:1, 20:1, 10:1, 5:1, 2:1, 1:1, 0:1) to give eleven subfractions (I–XI). Subfraction III was isolated and purified in a combination of silica gel, Sephadex LH-20, and preparative HPLC to afford compounds **1** (3.6 mg) and **2** (102.8 mg). The PE fraction (100.8 g) was fractionated by column chromatography to afford **3** (18.0 mg), **4** (10.0 mg), **5** (10.0 mg), **6** (28.5 mg), **7** (2113.3 mg), **8** (1144.4 mg), **9** (161.0 mg), and **10** (43.2 mg).

The IR spestrum of compound **1** showed absorption bands at ν_{max} 3408, 1608, 1570, and 1462 cm^{-1}. Its molecular formula was determined as $C_{10}H_{12}O_4$ on the basis of HR-ESI-MS at *m/z* 219.0630 $[M + Na]^+$ (calcd $C_{10}H_{12}O_4Na$, 219.0633), indicating five degrees of unsaturation. The similarity of the ^{13}C NMR spectra between **1** and 8,9,10-trihydroxythymol (**2**) [6] suggested that they were analogues. In fact, the main difference between them was the chemical shift of C-9 at δ_C 102.6 for **1**, in contrast to δ_C 66.9 for **2**. However, C-9 of **1** was confirmed as a methine by DEPT NMR. Furthermore, considering δ_C 102.6 and δ_H 5.70 (1H, s), the C-9 was believed to be at the junction of two oxygen atoms and a quaternary carbon atom, and the degree of unsaturation showed that there may be a small ring as well as a benzene ring. Thus, it comfirmed that **1** had a three-membered ring (8,9-epoxy ring). Therefore, the structure of **1** was assigned as 9,10-dihydroxy-8,9-epoxythymol.



1



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2: $R_1 = R_2 = H$
3: $R_1 = H$, $R_2 = COCH(CH_3)_2$
4: $R_1 = R_2 = COCH(CH_3)_2$
5: $R_1 = R_2 = CO(CH_3)C_2H_5$

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Compound 1. Colorless oil, $[\alpha]_D^{20}$ +11.3° (*c* 0.10, CH_3OH), ESI-MS *m/z* 219 $[M + Na]^+$, 195 $[M - H]^-$. ^1H NMR (500 MHz, $CDCl_3$:CD_3OD, 1:1, δ, ppm, J/Hz): 7.19 (1H, d, J = 7.5, H-5), 6.76 (1H, br.d, J = 7.5, H-6), 6.64 (1H, br, H-2), 5.70 (1H, s, H-9), 3.84 (1H, d, J = 11.6, H-10) , 3.64 (1H, d, J = 11.6, H-10), 2.31 (3H, s, 7-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$:CD_3OD, 1:1, δ, ppm): 141.6 (C-1), 114.4 (C-2), 158.6 (C-3), 125.4 (C-4), 124.8 (C-5), 122.6 (C-6), 22.0 (C-7), 79.6 (C-8), 102.6 (C-9), 66.7 (C-10).

8,9,10-Trihydroxythymol (2). Colorless oil, ESI-MS *m/z* 221 $[M + Na]^+$, 197 $[M - H]^-$. ^1H NMR (500 MHz, CD_3OD, δ , J/Hz): 7.16 (1H, d, J = 8.0, H-5), 6.62 (1H, dd, J = 8.0, 0.8, H-6), 6.58 (1H, d, J = 0.8, H-2), 3.85 (4H, m, 2H-9, 2H-10), 2.22 (3H, s, 7-CH_3); ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 134.0 (C-1), 118.2 (C-2), 157.4 (C-3), 124.8 (C-4), 128.9 (C-5), 121.5 (C-6), 21.3 (C-7), 80.2 (C-8), 66.9 (C-9, C-10) [7].

8,10-Dihydroxy-9-isobutyryloxythymol (3). Colorless oil, ESI-MS *m/z* 291 $[M + Na]^+$; 267 $[M - H]^-$. ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.16 (1H, d, J = 8.0, H-5), 6.64 (1H, dd, J = 8.0, 1.0, H-6), 6.60 (1H, d, J = 1, H-2), 4.56 (1H, d, J = 11.0, 9-CH_2), 4.40 (1H, d, J = 11.0, 9-CH_2), 3.91 (1H, d, J = 11.5, 10-CH_2), 3.84 (1H, d, J = 11.5, 10-CH_2), 2.49 (1H, m, H-2′), 2.23 (3H, s, 7-CH_3), 1.06 (3H, d, J = 7.0, 3′-CH_3), 1.03 (3H, d, J = 7.0, 4′-CH_3); ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 139.9 (C-1), 118.0 (C-2), 156.6 (C-3), 123.9 (C-4), 128.6 (C-5), 121.1 (C-6), 21.0 (C-7), 78.7 (C-8), 68.2 (C-9), 66.6 (C-10), 178.8 (C-1′), 35.1 (C-2′), 19.2 (C-2′), 19.1 (C-4′) [6].

8-Hydroxy-9,10-diisobutyryloxythymol (4). Colorless oil, ESI-MS *m/z* 361 $[M + Na]^+$, 337 $[M - H]^-$; ^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.91 (1H, d, J = 8.0, H-5), 6.69 (1H, br.s, H-2), 6.65 (1H, br.d, J = 8.0, H-6), 4.46 (4H, dd, J = 19.0, 11.9, H_2-9, H_2-9′), 2.56 (2H, m, H-11, H-11′), 2.27 (3H, s, 7-CH_3), 1.12 (12H, d, J = 7.0, 12, 12′, 13, 13′-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 140.0 (C-1), 118.5 (C-2), 156.4 (C-3), 119.0 (C-4), 126.5 (C-5), 120.5 (C-6), 20.9 (C-7), 78.5 (C-8), 76.2 (C-9, 9′), 177.5 (C-10, 10′), 33.9 (C-11, 11′), 18.8 (C-12, 12′, 13, 13′) [6].

8-Hydroxy-9-[(isobutyryl)oxy]-10-(2-methylbutanoyl)thymol (5). Colorless oil, ESI-MS *m/z* 375 $[M + Na]^+$, 351 $[M - H]^-$. ^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.89 (1H, d, J = 8.0, H-5), 6.70 (1H, br.s, H-2), 6.64 (1H, br.d, J = 8.0, H-6), 4.45 (4H, m, H_2-9, H_2-9′), 2.27 (3H, s, 7-CH_3), 2.56 (1H, m, H-11), 2.40 (1H, m, H-11′), 1.62 (1H, m, H-13′), 1.44 (1H, m, H-13′), 1.13 (6H, d, J = 7.0, 12, 13-CH_3), 1.10 (3H, d, J = 7.0, 12′-CH_3), 0.83 (3H, m, 14′-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 140.1 (C-1), 118.7 (C-2), 156.7 (C-3), 118.7 (C-4), 126.5 (C-5), 120.5 (C-6), 21.0 (C-7), 78.9 (C-8), 67.3 (C-9), 177.5 (C-10), 33.9 (C-11), 18.8 (C-12,13), 67.4 (C-9′), 177.2 (C-10′), 41.0 (C-11′), 16.5 (C-12′), 26.6 (C-13′), 11.43 (C-14′) [6].

Ursolic acid (6). White powder, ESI-MS *m/z* 479 $[M + Na]^+$, 455 $[M - H]^-$. ^{13}C NMR (125 MHz, C_5D_5N, δ, ppm): 27.8 (C-2), 77.8 (C-3), 39.1 (C-4), 55.5 (C-5), 18.5 (C-6), 33.3 (C-7), 39.7 (C-8), 47.7 (C-9), 37.0 (C-10), 23.6 (C-11), 125.3 (C-12), 138.9 (C-13), 42.2 (C-14), 28.4 (C-15), 24.6 (C-16), 47.7 (C-17), 53.2 (C-18), 39.2 (C-19), 38.8 (C-20), 37.1 (C-22), 28.5 (C-23), 15.3 (C-24), 16.2 (C-25), 17.2 (C-26), 23.3 (C-27), 179.5 (C-28), 17.1 (C-29), 21.1 (C-30) [8].

***β*-Sitosterol (7)**. White needle crystals, mp 124–125°C. The physical data of compound **7** are consistent with that of *β*-sitosterol [9] and showed the same color and equal R_f value as a standard substance of *β*-sitosterol when both compounds were applied on TLC. Furthermore, the melting point of the mixture of **7** and *β*-sitosterol did not decrease.

Daucosterol (8). White needles, mp 275–276°C. Compound **8** showed the same color and equal R_f value as a standard substance of daucosterol when applied on TLC and eluted with different developing solvents [10].

Stigmasterol-3-*O*-*β*-D-glucopyranoside (9). White powder, ESI-MS *m/z* 575 $[M + H]^+$. ^{13}C NMR (125 MHz, DMSO, δ, ppm): 33.3 (C-1), 29.2 (C-2), 76.7 (C-3), 36.8 (C-4), 140.4 (C-5), 121.1 (C-6), 31.3 (C-7), 35.5 (C-8), 49.6 (C-9), 36.8 (C-10), 23.8 (C-11), 38.3 (C-12), 41.8 (C-13), 56.2 (C-14), 25.4 (C-15), 28.4 (C-16), 55.4 (C-17), 28.7 (C-18), 31.3 (C-19), 43.7 (C-20), 31.4 (C-21), 138.0 (C-22), 128.8 (C-23), 50.6 (C-24), 21.0 (C-25), 20.6 (C-26), 18.8 (C-27), 19.7 (C-28), 11.8 (C-29), 100.8 (C-1′), 73.4 (C-2′), 76.9 (C-3′), 70.1 (C-4′), 76.7 (C-5′), 61.1 (C-6′) [11].

***α*-Monopalmitin (10)**. White powder. EI-MS *m/z* 330 $[M]^+$; ^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.21 (1H, dd, J = 12.0, 5.0, H-1), 4.15 (1H, dd, J = 12.0, 6.0, H-1), 3.94 (1H, m, H-2), 3.70 (1H, dd, J = 11.0, 4.0, H-3), 3.61 (1H, dd, J = 11.0, 5.5, H-3), 2.36 (2H, t, J = 7.5, 2H-5), 1.62 (2H, m, 2H-6), 1.29 (24H, m, 2H-7–18), 0.88 (3H, t, J = 7.0, 19-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 65.1 (C-1), 70.2 (C-2), 63.3 (C-3), 173.1 (C-4), 34.1 (C-5), 24.9 (C-6), 29.3 (C-7-16), 31.9 (C-17), 22.7 (C-18), 14.1 (C-19) [12].

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