SHORT REPORT

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Monoterpene Flavonoid from Aerial Parts of *Satureja khuzistanica*

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Abstract: Fractionation of methanolic extract of *Satureja khuzistanica* Jamzad by Sephadex LH-20 and reverse phase chromatography led to the isolation and purification of a new monoterpene flavonoid (**1**), as well as six previously detected flavonoid derivatives (**2**–**6**). The structure assignment has been performed by using 1D, 2D NMR, and high-resolution MS spectrometry. In addition, electronic circular dichroism (ECD) spectroscopy was used to reveal the absolute configuration of **1**.

Keywords: Lamiaceae; monoterpene flavonoid; structure elucidation; ECD.

1. Plant Source

The plant material of *Satureja khuzistanica* Jamzad was provided from the field of Khoraman Pharmaceutical Company, Khorramabad, Iran (2012), and was recognized by Dr. Javad Hadian. In addition, a voucher specimen (MPH-1582) was deposited at the Herbarium of the Department of Biology, Medicinal Plants, and Drugs Research Institute affiliated with Shahid Beheshti University (Tehran, Iran).

2. Previous Studies

The *Satureja* genus belongs to Lamiaceae family and contains over 30 species. The plants are mostly aromatic herbs distributed in the Mediterranean area, Middle East, North Africa, and Central Asia. Some of these species such as *Satureja montana*, *S. atropatana* and *S. hortensis* have been used as flavoring agents, herbal teas, and food additives [1,2]. In central part of Iran, the local people have been used the aerial part of *S. khuzistanica* for medical practitioners, analgesic, antibacterial, and antifungal purposes [3-5]. The essential oil composition of *S. khuzistanica* and its biological activities have been studied comprehensively [2-4]. Nevertheless, there are a few reports on the phytochemical profile of non-volatile constituents. A review of the literature revealed that rosmarinic acid, flavonoids, and triterpenoids are main compounds of *S. khuzistanica* aerial parts [6-8].

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3. Present Study

Dried leaf material of *Satureja khuzistanica* (800 g) was ground, and then extracted through successive percolation with CH_2Cl_2, EtOAc, and MeOH (3x5 L each). After evaporation to dryness under reduced pressure, 60 g of MeOH extract obtained. Then the extract was dissolved in distilled water (1L) passed through a Diaion HP-20 resin column (9 × 45 cm).The column rinsed with distilled water (3 × 1.5L). The enriched phenolic fraction obtained by rinsing the column with MeOH (3 × 1L) and evaporated under vacuum to give 15.0 g gummy material. The obtained enriched polyphenols dissolved in 100 ml MeOH and after filtration subjected to gel chromatography on Sephadex LH-20 column (55 × 880 mm). In the next stage, the column eluted with MeOH with a flow rate of 2.5 ml/min. 180 fractions were collected and monitored by TLC chromatography (EtOAc: MeOH, 8:2, detection at 254 and 360 nm), and were pooled into twelve major fractions (Fr 1-12). Fraction 5 (400 mg) was exposed to flash chromatography using a silica cartridge (Puriflash C18-HP 15µm, 40g), Interchim, Montluçon, France) to provide 13 subfractions (F5-1to F5-13). Subfraction F5-11 (5.0mg) was separated by a semi-preparative RP-HPLC by using a gradient of H_2O /MeCN (both with a 0.1% formic acid content) (80:20 to 50:50 over 26 min until it reached 100 % MeCN in 10 min) at a flow rate of 4.0 mL/min to afford compound **2** (0.8 mg, t_R 19.0 min), **3** (1.1 mg, t_R 22.0 min), **1** (0.3 mg, t_R 25.0 min), and **4** (0.3 mg, t_R 27.0 min). The reverse phase chromatography of subfraction F5-7 by a semi-preparative HPLC by the above method afforded compounds **5** (0.9 mg, t_R 29.0 min), and 6 (0.8 mg, t_R 32.0 min).

Compound **1**, yellowish powder; ECD (c=0.8 mM, MeOH, 25 °C): λ(Δε) = 224 (+4897), 258 (+1326), 296 (-3084), 332 (+1118). UV (MeOH):217(3.9), 278 (3.5), 335(3.6); HR-ESI-MS *m/z* = 451.1375 $[M–H]^-$: (Calcd 451.1398 for $C_{25}H_{24}O_8$). ^{1}HNMR (DMSO-d6, 500.13 MHz): δ 1.13 (6H, d, J= 6.9 Hz, Me-10″,Me- 9″), 2.04 (3H, s, Me-7″), 3.09 (1H, sep, J= 6.9 Hz, H-8″), 4.99 (1H, d, J= 11.0 Hz, H-2), 4.45 (1H, d, J= 11.0 Hz, H-3), 5.81 (1H, brs, H-6), 5.85 (1H, brs, H-8), 6.52 (1H, d, *J*= 8.3 Hz, H-5′), 6.55 (1H, s, H-6″), 6.75 (1H, s, H-3″), 6.81 (1H, dd, J= 8.3, 1.9 Hz, H-6′), 7.09 (1H, d, J= 1.9 Hz, H-2′); ^{13}C NMR (125.1 MHz, DMSO-d6): δ 15.8 (C-7″), 23.3 (C-10″ and C-9″), 26.4 (C-8″), 71.9 (C-3), 82.9 (C-2), 95.9 (C-6), 96.6 (C-8), 151.0(C-4′), 100.5 (C-10), 112.9 (C-3″), 147.3 (C-3′), 119.2 (C-6′), 116.5 (C-2′), 120.6 (C-6″), 123.6 (C-1″), 132.2 (C-1′), 138.5 (C-4″), 145.3 (C-5″), 116.5 (C-5′), 151.9 (C-2″), 159.1 (C-9), 166.7 (C-7), 197.1 (C-4).

The phytochemical profiling of methanolic extract of *S. khuzistanica* carried out by partitioning using diaion HP-20 resin, Sephadex LH-20, and preparative and semi-preparative reverse phase chromatography. A new monoterpene flavonoid plus five known flavonoid derivatives (Figure 1) were identified through the isolation and purification of selected fractions. The structure of these compounds was depicted by means of 1D and 2D NMR spectroscopy and the comparison of those published data. The identified compounds were: keshonin (**2**) [12], saturejin (3′-(2,5-dihydroxy-p-cymene) 5,7,4′-trihydroxyflavone) (**3**) [6], ponciretin (**4**) [13], 5,6-dihydroxy-3′,4′,7-trimethoxyflavone (**5**) [14], 5,6-dihydroxy-4′,7-dimethoxyflavone (**6**)[15], and acacetin (**7**) [6]. Compounds **2–5** were the first isolated from *S. khuzistanica*.

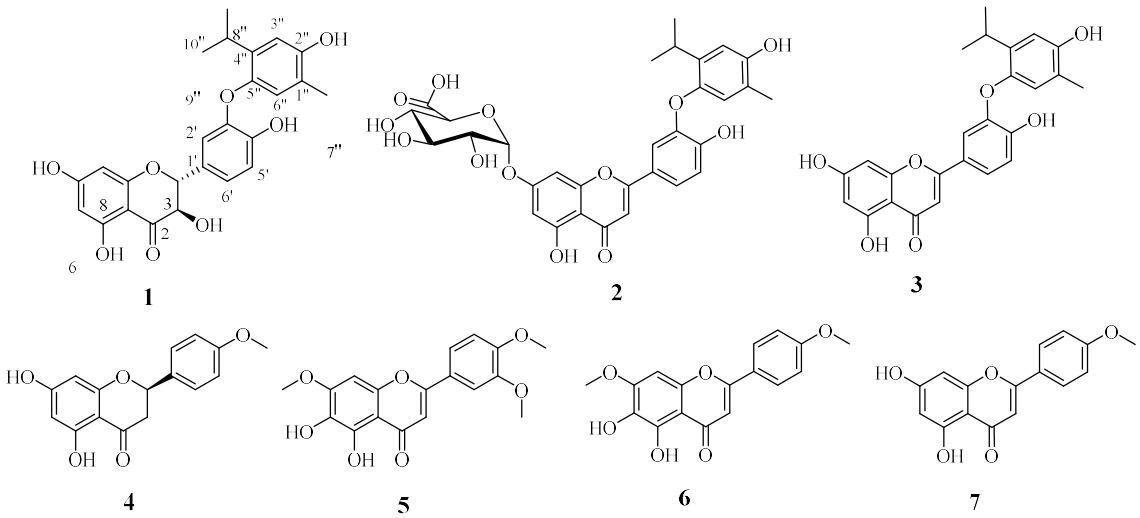

Figure 1. Structures of isolated compounds from *S. khuzistanica*

Compound **1** was obtained as a yellow amorphous powder with a molecular formula of $C_{25}H_{24}O_8$ from high-resolution TOF-MS (m/z 451.1375 [M–H]−: calculated 451.1398). It showed absorbance in UV spectroscopy at 278 and 335 nm, typical of flavonoids. In the 1H NMR spectrum, seven aromatic proton signals at δH 7.09(d, J = 1.9 Hz, H-2′), δH 6.81(dd, J = 8.3, 1.9 Hz, H- 6′), δH 6.75 (s, H- 3″), δH 6.55 (s, H-6″), δH 6.52(d, J = 8.3 Hz, H-5′), δH 5.85(brs, H-8), and δH 5.81(brs, H-6), together with methine proton signals at δH 4.45 (d, J = 11 Hz, H-3) and δH 4.99 (d, J = 11 Hz, H-2) suggested flavone 3-ol structure matched by taxifolin core. The signals for one methyl group at δH 2.04(s, Me-7″) and two methyl groups at δH 1.13 (d, J = 6.9 Hz, Me-9″ and 10″) that coupled with methyl group at δH 3.09 (sep, H-8″) showed the presence of p-cymene-2,5-diol moiety. The arrangement of the methyl group at 5″ was confirmed by HMBC correlation from H-7″ to C-1″ and C-2″. The cross peak in HMBC between H-8″, Me-9″ and Me-10″ to C-4″ confirmed the presence of thymoquinol moiety which is comparable to saturejin. The key cross-signal in NOESY spectrum between Me-9″ and Me-10″ to H-2′ on ring B suggested that thymoquinol moiety was attached to position 3′ [12]. The analysis of various spectral data indicated that this compound should be structured as monoterpene–flavonoid (3′-(2,5-dihydroxy-p-cymene),5,7,-trihydroxyflavol. Besides, the vicinal coupling constant between H-2/H-3 (J=11 Hz) corresponded with trans-orientation dihedral angel of about 170-180 degree. Therefore, two stereoisomer are possible 2*S*,3*S*/2*R*3*R*. The absolute configuration of position C-2 and C-3 deduced by comparison with those published data [16]. The experimental ECD spectrum of **1** displayed two positive Cotton effects (CE) at 332 and 215 nm and a negative CE at 296 nm (figure S13). A assessment of ECD spectrum showed the good agreement with 2*R*,3*R* configuration comparing to (+)-taxifloin, with sequential positive and negative CEs at 330 and 295 nm, respectively [16]. Therefore, compound **1** was a new natural product named as saturejenol (**1**).

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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