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Two flavones from *Scutellaria baicalensis* Georgi and their binding affinities to the benzodiazepine site of the $GABA_A$ receptor complex

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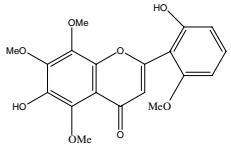
A new flavone 6,2′-dihydroxy-5,7,8,6′-tetramethoxyflavone (**1**) together with one known flavone 5,7,2′-trihydroxy-6,8-dimethoxyflavone (**2**) were isolated from the roots of *Scutellaria baicalensis* Georgi. Their structures were elucidated on the basis of spectral evidence and their affinities for the benzodiazepine (BDZ) site of the $GABA_A$ receptor complex were evaluated with a radioligand receptor binding assay.

1. Introduction

Scutellaria baicalensis Georgi is one of the most important medicinal herbs in traditional Chinese medicine and has shown diverse biological and pharmacological actions such as antibacterial, antiviral, and antioxidative activities and antitumor properties. We previously reported the isolation and $GABA_A$ receptor BDZ-site binding assay of several flavones from the roots of *Scutellaria baicalensis* Georgi [1]. In this paper, we report the isolation and structural elucidation of two flavones from the roots of *Scutellaria baicalensis* Georgi, and their affinities to the BDZ site of the $GABA_A$ receptor complex.

2. Investigations, results and discussion

Compound **1** was isolated as a white amorphous powder. It exhibited $[M-H]^-$ peak at *m/z* 373 ($C_{19}H_{18}O_8$) in its negative-ion electrospray ionization mass spectrum (ESI-MS) and showed the presence of a hydroxyl group (3374 cm^{-1}) and a carbonyl group (1629 cm^{-1}) in its IR spectrum. In EI-MS, the molecular ion was established as 374 and two fragment ions appearing at m/z 225 and 149, derived from *retro*-Diels-Alder fragmentation, suggested the presence of a hydroxyl group and three methoxyl groups in ring A and of a hydroxyl and a methoxyl group in ring B.



Compound **1**

The ^1H NMR spectrum (Table 1) of **1** showed four methoxyl, a non-coupled olefinic, ABC-type aromatic and two hydroxyl proton signals. Due to the lack of a chelated OH signal near δ 13.0 in the ^1H NMR and no shifts in the UV spectrum on addition of $AlCl_3$, one of four methoxyl groups should be located at C-5. This signal pattern indicated that 1 has a 5 (OMe), 6, 7, 8, 2′, 6′-pentahydroxyflavone structure.

Table 1: ^1H NMR spectral data of 1 (in DMSO-d_6)

No.	1	No.	1
3	6.06 (s)	2′-OH	10.03 (s)
5-OMe	3.74 (s)	3′	6.62–6.59 (d, 8)
6-OH	9.27 (s)	4′	7.32–7.26 (t, 8)
7-OMe	3.94 (s)	5′	6.62–6.59 (d, 8)
8-OMe	3.82 (s)	6′	3.74 (s)

Table 2: ^{13}C NMR spectral data of 1, 1a and 1b (in DMSO-d_6)

No.	1	1a	1b
2	158.4	160.1	162.0
3	114.3	105.7	114.4
4	175.9	175.9	175.5
5	141.2	141.2	143.2
6	140.8	140.7	137.4
7	146.4	146.3	150.7
8	137.8	137.8	137.4
9	144.8	143.6	147.3
10	114.3	114.1	114.4
1′	109.4	123.2	108.6
2′	156.6	127.6	156.4
3′	108.8	114.5	106.1
4′	132.0	161.8	131.8
5′	102.3	114.5	102.1
6′	158.4	127.6	158.2
5-OMe	61.7	61.7	61.4
7-OMe	61.0	61.4	61.4
8-OMe	61.5	60.8	61.4
6′-OMe	55.9	55.3	55.7

The ^{13}C NMR spectrum of **1** (Table 2) exhibited 17 carbons whose A-ring carbon chemical shifts were in good agreement with those of 6-hydroxy-5,7,8,4′-tetramethoxyflavone (**1a**) [2] and whose B-ring carbon chemical shifts were in good agreement with those of 2′-hydroxy-5,6,7,8,6′-pantamethoxyflavone (**1b**) [3]. This structural assignment was further confirmed by HMBC experiments which showed long range correlations between C-6-OH (δ 9.27) and C-5 (δ 141.5), C-7 (δ 146.4) and C-6 (δ 140.8) and between C-2′-OH (δ 10.03) and C-1′ (δ 109.4), C-3′ (δ 108.8) and C-2′ (δ 156.6). Based on these data, it was concluded that compound **1** is 6,2′-dihydroxy-5,7,8,6′-tetramethoxyflavone.

On the basis of spectral analysis, compound **2** was identified as 5,7,2′-trihydroxy-6,8-dimethoxyflavone [4].

Compound **1** showed weak affinity to the BDZ site with a K_i of 32.77 ± 1.5 μmol/l, whereas compound **2** significantly inhibited [^3H] flunitrazepam binding to the central benzodiazepine receptors (BDZ-Rs) with a K_i of 1.05 ± 0.2 nM, which affinity is slightly higher even than the commercial drug diazepam.

3. Experimental

3.1. Equipment

ESI-MS was recorded on a Finnigan LCQ LC/ESI-MS spectrometer. IR spectra were recorded on a Bruker IFS-55. ^{1}H and ^{13}C NMR, togetherg with 2D NMR (HMQC and HMBC) spectra were taken on a Bruker ARX-300 FT NMR spectrometer (^{1}H at 300 MHz and ^{13}C at 75 MHz) in DMSO. CC was performed on silica gel (Merck, 70-230 mesh) and polyamide 6S (Riedel-deHaen, Germany). TLC analysis was carried out on silica gel 60 (Merck).

3.2. Plant material

The roots of *Scutellaria baicalensis* were purchased from the Hong Kong market as a dried crude herb and identified by Prof. Y. Z. Guo, Faculty of Traditional Chinese Medicine, Shenyang Pharmaceutical University, P. R. China. A voucher specimen (SB1) is deposited in the Department of Biochemistry, The Hong Kong University of Science and Technology.

3.3. Extraction and isolation

The roots of *Scutellaria baicalensis* Georgi (5 kg) were extracted three times with dichloromethane at room temperature. The extracted solutions were evaporated at reduced pressure. A portion (50 g) of the CH_2Cl_2 extract was subjected to CC on silica gel (600 g, 70–230 mesh) with $CHCl_3$-MeOH gradients (100 : 1–2 : 1) as elute to give fractions 1–450. Fractions 355–395 (2 g) were subjected to CC on polyamide 6S eluted by $CHCl_3$-MeOH gradients (100 : 1–10 : 1) to give compound **1** (6 mg) and compound **2** (8 mg).

6,2′-dihydroxy-5,7,8,6′-tetramethoxyflavone (**1**): White amorphous powder. EI-MS *m/z* 374 M^+. ESI-MS m/z: 373 $[M-H]^-$. UV λ_{max}^{MeOH} 264.6 nm. IR ν_{max}^{KBr} cm^{-1}: 3374, 1629, 1468, 1436. For ^{1}H and ^{13}C NMR data see Tables.

3.4. Receptor binding assay

Compounds **1** and **2** were dissolved in DMSO and assayed at less than 0.2% final DMSO concentration. DMSO itself at a concentration less than 0.5% showed no significant effects on the BZD-S assay. The radioreceptor BZD-S assay was performed as previously described [5]. Whole forebrains from decapitated Sprague-Dawley rats (approximately 250 g) were homogenized to give the crude synaptosomal fraction. In the assay procedure, 45 µl of the crude synaptosomal fraction (0.8 mg/ml) were added to an incubation mixture containing 145 µl 0.05 M Tris-HCl (pH 7.4) and 84 µl water, with or without the test compound. Samples were incubated in duplicate for 2 h at 4 °C. In the competition assay, 3 nM [^{3}H] flunitrazepam (Amersham, Hong Kong) was employed; in the saturation assay, twelve concentrations (1.33-125 nM of [^{3}H] flunitrazepam were employed, and non-specific binding was determined by the addition of 100 µM diazepam to calculate specific binding. After incubation, the reaction was rapidly stopped by filtration through a Millipore GF/C filter and washing twice with 5 ml ice-cold 0.05 M Tris-HCl buffer before drying. IC_{50} values were expressed as means ± S.E.M.

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