Flavonoid Aglycones from Transformed Root Culture of ڑ

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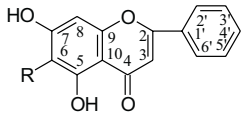
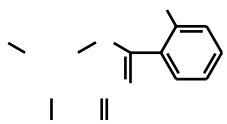
Flavones unsubstituted in the B-ring, such as chrysin (**1**), baicalein (**2**), oroxylin A (**3**), norwogonin (**4**) and wogonin (**5**), are widely distributed in *Scutellaria* species (Lamiaceae) [1]. Long-known source of the compounds is the root of *Scutellaria baicalensis* Georgi, a famous remedy in Chinese and Japanese traditional medicine. Over 40 flavonoid aglycones and glycosides have been isolated from this plant material, including 7-O-glucuronides of **2**, **3** and **5** as major constituents. In our previous paper [2] we have reported on the capability of *S. baicalensis* roots, transformed with *Agrobacterium rhizogenes* LBA 9402, to produce similar amounts of major flavonoids to those of roots of the intact plant. Three flavonoid aglycones (**2**, **3** and **5**) were quantified by HPLC after acid hydrolysis of glycosides present in the root extracts.

In this study emphasis was placed on the isolation of the resulting flavonoid aglycones from the transformed root hydrolysate. A crude mixture of the compounds was subjected to column chromatography on silica gel followed by semipreparative HPLC to afford the above mentioned compounds **1–5**, along with 5,7,2′-trihydroxyflavone (**6**) [3], 5,2′-dihydroxy-6,7,8-trimethoxyflavone (**7**) [4] and 5,2′,6′-trihydroxy-6,7,8-trimethoxyflavone (**8**



1 R = H
2 R = OH
3 R = OCH_3



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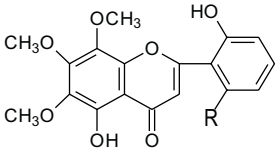

7 R = H
8 R = OH

comparative studies of their ^{1}H NMR spectra and reported data. The spectra were recorded in DMSO-d_6, the most effective general solvent for NMR spectroscopy of flavonoid aglycones and glycosides.

A search of the literature revealed that the C-3 proton signal assignments in the reported ^{1}H NMR spectra of **2** (δ 6.63), **3** (δ 6.62) and **5** (δ 6.31) in DMSO-d_6 were not compatible with that of **1** (δ 6.92) [6]. Moreover, we could not find ^{13}C NMR data of the compounds in DMSO-d_6, except for those of **5** [7], for comparison. The proton and carbon signal assignments of **1–3** and **5** (Tables 1 and 2) were based on HETCOR analysis and tabulated ^{13}C NMR data of 4′-methoxyflavones variously substituted in the A-ring [8]. It was evident from this study that the ^{1}H and ^{13}C NMR assignments required some corrections in previously published data, *i.e.* interchanging of the assignments of H-3 and the A-ring proton signals (H-8 or H-6) of **2**, **3** and **5** [6, 9], as well as those of C-5, C-7 and C-9 of **5** [7, 9].

Table 1. ^{1}H NMR spectral data of compound **1–3** and **5**[a].

H	**1**	**2**	**3**	**5**
3	6.97 *s*	6.93 *s*	6.99 *s*	7.00 *s*
6	6.22 *d* (2.0)	–	–	6.32 s
8	6.53 *d* (2.0)	6.63 *s*	6.65 *s*	–
2′, 6′	8.06 *dd* (8.5, 1.5)	8.06 *dd* (8.4, 1.6)	8.08 br *d* (8.2)	8.07 br *dd* (8.2, 1.4)
3′, 4′, 5′	7.60 *m*	7.59 *m*	7.59 *m*	7.62 *m*
5-OH	12.83 *s*	12.66 br *s*	12.94 *s*	12.50 *s*
7-OH	10.91 br *s*	10.57 br *s*	10.82 br *s*	10.84 br *s*
-OCH_3	–	–	3.76 *s*	3.86 *s*

[a] Run in DMSO-d_6 at 500.13 MHz, δ–values, *J* (Hz) in parentheses.

Table 2. ^{13}C NMR spectral data of compounds **1–3** and **5**[a].

C	**1**	**2**	**3**	**5**
2	163.13	162.90	163.16	162.97
3	105.15	104.65	104.65	105.03
4	181.83	182.11	182.25	182.01
5	161.43	146.95	152.73*	156.19
6	98.98	129.31	131.46	99.12
7	164.40	153.62	157.68	157.36
8	94.08	94.00	94.39	127.75
9	157.42	149.82	152.54*	149.58
10	103.94	104.26	104.33	103.72
1′	130.68	130.72	130.69	130.82
2′	126.37	126.39	126.26	126.24
3′	129.09	129.10	129.24	129.22
4′	131.96	132.00	132.00	132.04
5′	129.09	129.10	129.24	129.22
6′	126.37	126.29	126.26	126.24
-OCH_3	–	–	59.94	61.02

[a] Run in DMSO-d_6 at 125.76 MHz, the chemical shifts confirmed by HETCOR correlations.
* Values interchangeable.

General procedure: Column chromatography: silica gel Merck Art.7754; semipreparative HPLC: Delta-Pak C18 cartridge column Waters No 38506 (particle size 15 μm, 25 mm × 100 mm) coupled to a UV photodiode array detector with MeOH – 0.025% H_3PO_4 (pH 2.4) systems as mobile phase.

Plant material: Roots of *Scutellaria baicalensis* Georgi, transformed with *Agrobacterium rhizogenes* LBA 9402, were obtained and cultivated as described elsewhere [2]. The roots grown in MS nutrient medium [10] containing $^1/_2$ strength macronutrients and 3% sucrose were harvested four weeks after the last transfer to the fresh medium.

Extraction and acid hydrolysis of flavonoid glycosides: The lyophilized and powdered roots (3.7 g) were suspended in 740 ml of 2N HCl and refluxed at 100° C for 3 h. The final hydrolysate was shaken with EtOAc till exhaustion to obtain a combined EtOAc extract containing flavonoid aglycones.

Isolation of flavonoid aglycones: The EtOAc solubles were evaporated to dryness providing a residue (0.8 g) which was chromatographed on a silica gel column sequentially eluted with hexane-EtOAc (up to 50% EtOAc) mixtures of increasing polarity and the relevant fractions were combined as shown by TLC. Elution of the column with hexane-EtOAc (9:1) afforded **3** (12.8 mg), a mixture of **3** and **1** (*ca* 5:1, respectively, 12.7 mg), a mixture of **3**, **1** and **5** (*ca* 4:1:15, respectively, 15.7 mg) and a mixture of **1** and **5** (*ca* 1:17, respectively, 13.4 mg). Fractions eluted with hexane-EtOAc (8:2) were further separated by semipreparative HPLC using MeOH – 0.025% H_3PO_4 (2:1) mixture at a flow rate of 4 ml min^{-1} to yield **7** (Rt – 58 min, 9.8 mg) and additional amounts of **3** (Rt – 49 min, 3.2 mg) and **5** (Rt – 39 min, 4.6 mg). More polar fractions from hexane-EtOAc (1:1) elution were rechromatographed as above using MeOH – 0.025% H_3PO_4 (3:2) mixture at a flow rate of 5 ml min^{-1} to afford **8** (Rt – 18 min, 13.2 mg), **4** (Rt – 31 min, 35.0 mg), **2** (Rt – 38 min, 40.9 mg), **6** (Rt – 50 min, 8.4 mg) and **5** (Rt – 68 min, 4.3 mg). The flavonoid mixtures, indicated by ^{1}H NMR, were not separated further as the ^{1}H NMR signals could be readily assigned to the respective compounds on the basis of their relative amounts.

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