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CONFIRMING THE CHEMICAL STRUCTURE OF ANTIOXIDATIVE TRIHYDROXYFLAVONES FROM *SCUTELLARIA BAICALENSIS* USING MODERN SPECTROSCOPIC METHODS

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Key words: trihydroxyflavones of *Scutellaria baicalensis*, high-performance liquid chromatography, baicalin and baicalein, structure elucidation

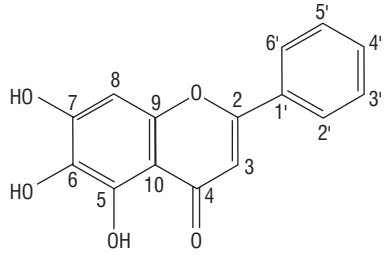
Phenolics were extracted from the roots of *Scutellaria baicalensis* using methanol. The compounds 5,6,7-trihydroxyflavone and 5,6,7-trihydroxyflavone-7-O-β-D-glucopyrano-siduronate, which are commonly referred to as baicalein and baicalin, respectively, were isolated from the crude extract using a semi-preparative HPLC method on a RP-18 column. The identities of the separated trihydroxyflavones were determined by electron impact (EI), chemical ionisation (CI) and/or fast atom bombardment (FAB) mass spectrometry and confirmed by ^{1}H, $^{13}C\{^{1}H\}$, HMQC and HMBC-NMR spectral evidence to be baicalein and baicalin.

ABBREVIATIONS

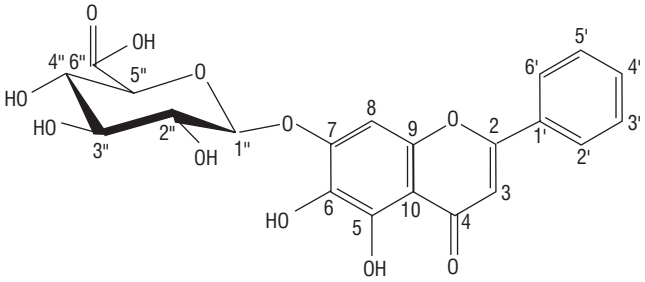
* – chemical shift in parts-per-million units; br – broad peak [in NMR spectroscopy]; $^{13}C\{^{1}H\}$– carbon-13 spectra, which is broadband decoupled [in NMR spectroscopy]; CI – chemical ionisation; d – doublet [in NMR spectroscopy]; dd – doublet of doublets [in NMR spectroscopy]; DMSO-d_6 – deuterated dimethyl sulphoxide; D_2O – deuterated water; EI – electron impact; ex – exchangeable proton [in NMR spectroscopy]; FAB – fast atom bombardment; ^{1}H – proton [as in a proton NMR spectrum]; HETCOR – heteronuclear correlation [in NMR spectroscopy]; HDL – high density lipoprotein; HMBC – heteronuclear multiple bond coherence [in NMR spectroscopy]; HMQC – heteronuclear multiple quantum coherence [in NMR spectroscopy]; HPLC – high-performance liquid chromatography; *J* – coupling constant [in NMR spectroscopy]; m – multiplet [in NMR spectroscopy]; *m*/*z* – mass-to-charge ratio; NMR – nuclear magnetic resonance; RP-18- reversed-phase C_{18}; s – singlet [in NMR spectroscopy]; TBARS – 2-thiobarbituric acid reactive substances; UV – ultraviolet.

INTRODUCTION

Dried roots of *Scutellaria baicalensis* are a very old, well-known drug in traditional Chinese herbal medicine for the treatment of bronchitis, hepatitis, diarrhoea and tumours [Tang & Eisenbrand, 1992; Zhou *et al.*, 1997]. These roots contain a number of biologically-active flavone derivatives: baicalein (5,6,7-trihydroxyflavone) and baicalin (5,6,7-trihydroxyflavone-7-O-β-D-glucopyranosiduronate) (Figure 1) are two such examples, which have been isolated from *S. baicalensis* and used clinically as therapeutic medicine in China [Chen *et al.*, 2000]. In experiments on rats fed diets supplemented with polyphenolic extracts from *S. baicalensis*, a marked decrease in the contents of HDL-cholesterol and triacylglycerols was observed by Zduńczyk *et al.* [2002].



5,6,7-trihydroxyflavone
(Baicalein – Compound 1)



5,6,7-trihydroxyflavone-7-*O*-β-D-glucopyranosiduronate
(Baicalin – Compound 2)

FIGURE 1. Chemical structure of flavones from *Scutellaria baicalensis*.

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Gabrielska *et al.* [1997a, 1997b] found that the trihydroxyflavones of *S. baicalensis*, namely baicalin, baicalein and wogonin, ensured a very satisfactory concentration-dependent protection of the liposome membrane against UV-induced oxidation. These authors reported that the antioxidative activity of an extract from *S. baicalensis* was similar to that of butylated hydroxytoluene, as confirmed in a test using an index of conjugated diene. Zhang and Shen [1997] reported that baicalin was a more effective antioxidant than green tea polyphenols and vitamin E in reducing the hepatic content of 2-thiobarbituric acid reactive substances (TBARS) and better than vitamin E in lowering the serum level of alanine aminotransferase activity in bromobenzene-intoxicated mice. Using a food system, Pegg *et al.* [2005] compared the TBARS values of cooked pork treated with an extract from *S. baicalensis* to meat systems treated with either a synthetic (*e.g.* tert-butylhydroquinone) or a natural antioxidant (*e.g.* a rosemary extract). Results confirmed the antioxidant efficacy of constituents from *S. baicalensis* in a thermally-processed food product. The antioxidant activity of the flavones may partially be due to metal ion chelation. Wybieralska *et al.* [2001] reported that the protective effect of baicalin against oxidation was attributable to the chelation of divalent metal cations.

MATERIAL AND METHODS

Material. Roots of *S. baicalensis* were obtained from the experimental field of the Agricultural University in Wrocław (Poland). Deuterated dimethyl sulphoxide (DMSO-d_6), sodium dodecyl sulphate (SDS) and heptakis(2,6-di-O-methyl)-β-cyclodextrin were obtained from Sigma-Aldrich Canada Ltd. (Oakville, ON). Deuterated water (D_2O) was acquired from Cambridge Isotope Laboratories (Andover, MA). Other chemicals were of analytical grade and purchased from VWR International (Mississauga, ON).

Extraction parameters. A 10 g portion of dry powdered roots of *S. baicalensis* was extracted three times with 100 mL of methanol for 15 min at 60°C [Amarowicz *et al.*, 1995]. The solution was then filtered through a Schott funnel with Whatman No. 1 filter paper and the residue was re-extracted twice more. Combined supernatants were evaporated to dryness under vacuum at 40°C using a Büchi Rotavapor/Water Bath (Models EL 131 and 461, respectively, Brinkmann Instruments [Canada] Ltd., Toronto, ON). Prepared extracts were stored at 4°C in air until further analysed.

Semi-Preparative High-Performance Liquid Chromatography (HPLC). Semi-preparative HPLC was carried out using a Waters HPLC system, which consisted of the following components: a Waters 600 controller, a 600E multisolvent delivery system, a 996 photodiode array detector, a 715 Ultra Wisp sample processor and Millenium software. Conditions of separation entailed a prepacked semi-preparative RP-18 column (5 μm, 10 × 250 mm; Agilent Technologies Canada Inc., Mississauga, ON), a mobile phase of methanol:water:acetic acid (12:7:1; v/v/v), a flow rate of 4 mL/min, an injection volume of 500 μL and the detector being set at 280 nm. Samples so obtained were dried *in vacuo* and then stored at 4°C in air until further analysed.

Mass Spectrometry. Mass spectra were recorded using a VG analytical mass spectrometer (Manchester, UK). Depending upon the nature of the compound, electron impact (EI), chemical ionisation (CI) and fast atom bombardment (FAB) modes were all employed. The conditions for EI were the following: ion source temperature, 200°C; ionisation energy, 70 eV; mass range, 50–850 amu; and pressure, 1×10^{-7} mbar. For CI, ammonia was used as the ionisation reagent and the mass spectral conditions were as follows: ion source temperature, 175°C; ionisation energy, 50 eV; mass range, 33–850 amu; and pressure, 5×10^{-5} mbar. For FAB, the compounds of interest were distributed on a matrix of 3-nitrobenzyl alcohol and a caesium-ion gun source was used. Positive identifications were based on authentic standard compounds.

Nuclear Magnetic Resonance (NMR) Spectroscopy. Nuclear magnetic resonance (NMR) spectra were obtained at 300 and 500 MHz on Bruker AMX-300 and Avance 500 (Bruker Analytik GmbH, Rheinstetten, Germany) spectrometers, respectively. ^{1}H and ^{13}C{^{1}H} NMR data were collected at room temperature in DMSO-d_6 or in DMSO-d_6/D_2O mixtures. Chemical shifts (δ) are reported as parts-per-million and referenced to the tetramethylsilane internal standard. In addition, *J*-modulated spin-echo, heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond coherence (HMBC) experiments were performed to help further elucidate the chemical structure of the compounds. The accompanying software packages, XWINNMR and XWINPLOT version 2.0, for the 500 MHz machine were used to process the data and plot the spectra.

RESULTS AND DISCUSSION

Semi-Preparative High-Performance Liquid Chromatography (HPLC)

Semi-preparative HPLC of the crude methanolic extract of *S. baicalensis* resulted in the detection of *ca.* 12 peaks at the 280 nm wavelength over a 40-min analysis period (Figure 2). The chromatogram showed two well-resolved dominant peaks, believed to be baicalein and baicalin, with retention times of 19.20 and 23.10 min, respectively. During subsequent runs of the extract, eluent was collected at the 19.20 and 23.10 min retention times, solvent removed *in vacuo* and products then labelled as compounds 1 and 2, respectively.

Structure elucidation

Compound 1

Mass Spectrometry. Compound 1 appeared as pale yellow amorphous powder. Its EI mass spectrum (Figure 3) showed the molecular ion radical, $M^{+\bullet}$, at an *m/z* of 270 (100% — relative intensity) and a $[M+H]^+$ peak at an *m/z* of 271 (16.3%). The CI mass spectrum (Figure 4), in which ammonia was used as the ionisation reagent, exhibited a $[M+H]^+$ peak at an *m/z* of 271 (100%), but no $[M+NH_4]^+$ signal at an *m/z* of 288. Mass spectra indicated that compound 1 has a molecular weight of 270 Da, which is characteristic of baicalein ($C_{15}H_{10}O_5$).

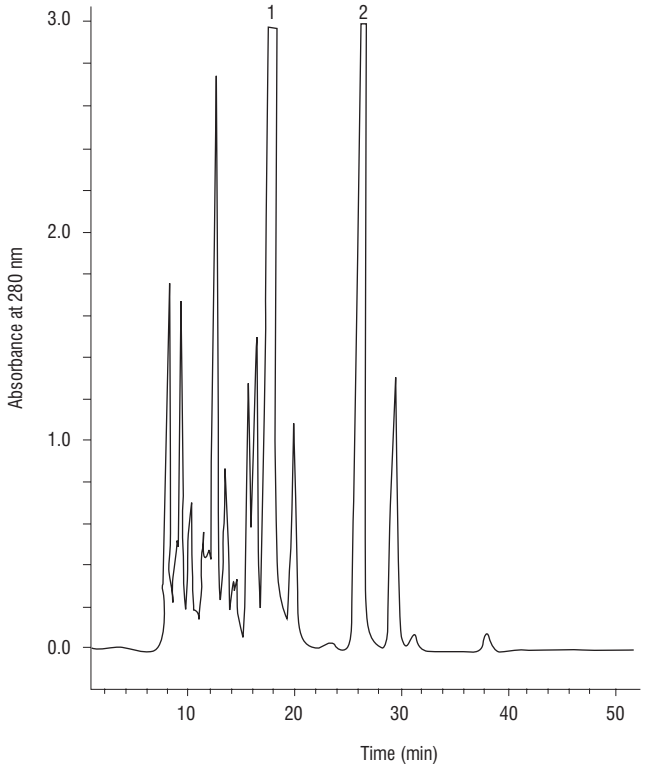

FIGURE 2. Semi-preparative HPLC chromatogram of a crude methanolic extract of *Scutellaria baicalensis*.

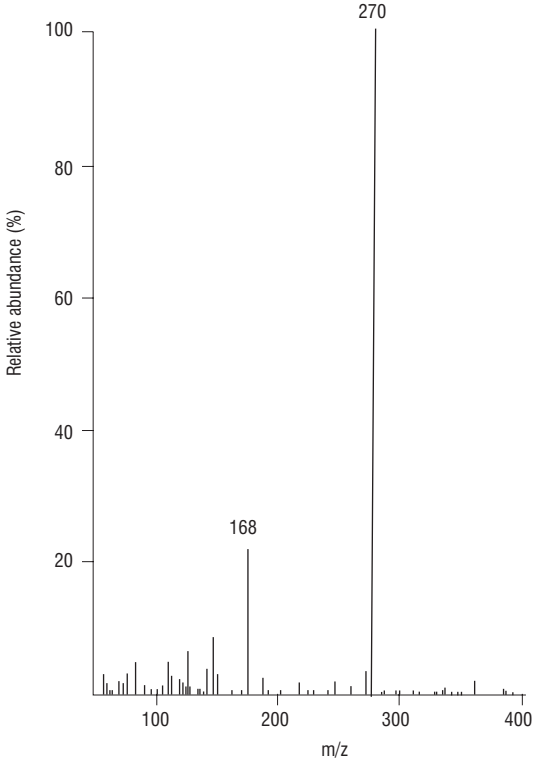

FIGURE 3. EI-MS spectrum of compound 1.

Nuclear Magnetic Resonance Spectroscopy Experiments. Based on chemical shifts (δ), integration and a deuterated water shake, the ^{1}H NMR spectrum of compound 1 (Figure 5) can be summarised as follows: δ (in ppm) 6.64 [s, 1H, H–C(8)], 6.93 [s, 1H, H–C(3)], 7.59 [m, 1H, H–C(3')],

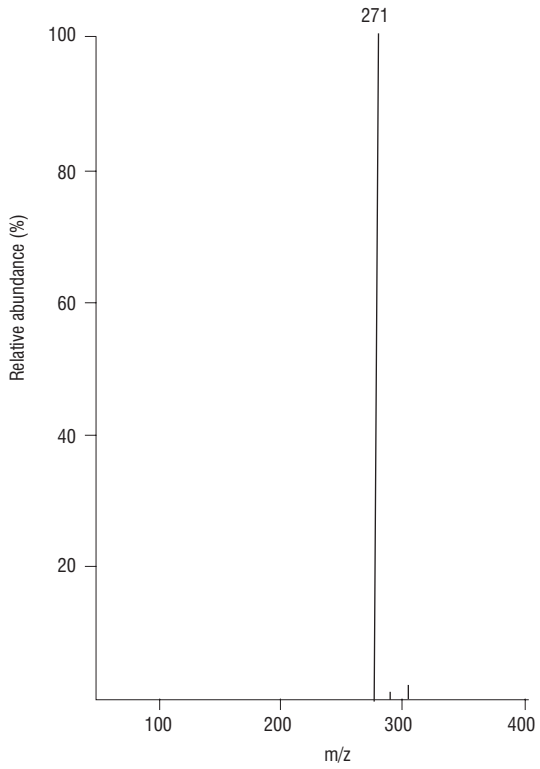

FIGURE 4. CI-MS spectrum of compound 1.

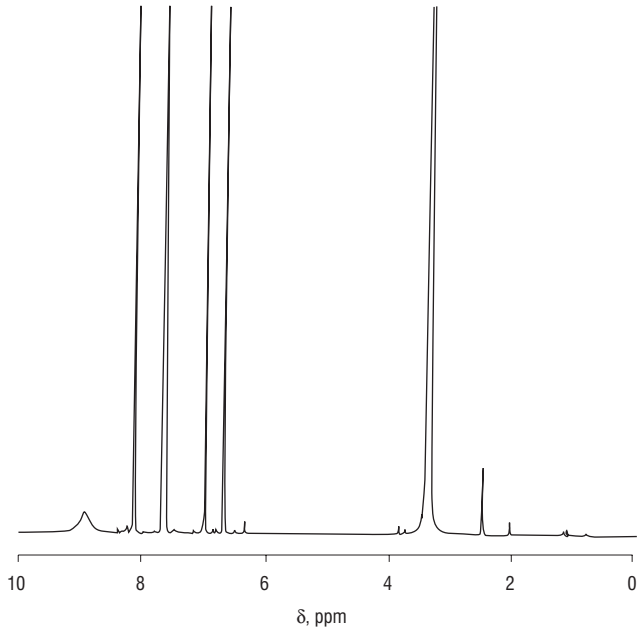

FIGURE 5. ^{1}H-NMR spectrum of compound 1.

7.59 [m, 1H, H–C(5')], 7.59 [m, 1H, H–C(4')], 8.06 [dd, 1H, $^{3}J_{2'3'}$=7.52 Hz, $^{4}J_{2'4'}$=1.51 Hz, H–C(2')], 8.06 [dd, 1H, $^{3}J_{6'5'}$=8.09 Hz, $^{4}J_{6'4'}$=2.08 Hz, H–C(6')], 8.84 [s (br), 1H, ex, OH], 10.58 [s (br), 1H, ex, OH], 12.65 [s, 1H, ex, HO–C(5)]. The δ at 6.93 ppm was in the range expected for an olefinic hydrogen atom and due to the lack of vicinal hydrogens, the signal appeared as a singlet. The δ at 6.64 ppm also appeared as a singlet, but is part of the A-ring. Two sets of protons in the B-ring (*i.e.* 2' & 6' and 3' & 5') are chemically, but not magnetically equivalent; thus, the five protons of the B-ring are behaving somewhat like a five-spin system.

The doublet of doublets signal centred at 8.06 ppm is characteristic for the aromatic protons on the B-ring at 2' & 6'. The *ortho* and *meta* coupling constants for these protons deviate from one another by approximately 0.5 Hz. The electronic environment of the proton at 6' could be slightly affected by the ether oxygen of the C-ring, based on the favoured spatial arrangement of the molecule. Hence, a slight difference in the *ortho* and *meta* coupling constants might be observed. The 1H signals of the aromatic protons at 3', 4' and 5' showed a multiplet; the individual coupling constants for protons *ortho* (*e.g.*, $^{3}J_{4'5'}$) and *meta* (*e.g.* $^{4}J_{4'6'}$) at each position could not be determined due to the complexity of the spectrum. Nevertheless, integration of this multiplet revealed that 3 protons were present at the signal centred at δ 7.59 and assignments for these were made. The δ at 12.65 ppm is characteristic of a chelated hydroxyl proton; that is HO–C(5). Flavones are known to chelate metal ions at the 5-hydroxy-4-keto group when the A-ring is hydroxylated at the fifth position [Cuppett *et al.*, 1997].

The $^{13}C\{^{1}H\}$ NMR spectrum (Figure 6) showed 13 distinct signals arising from one flavone (15 carbons, C–2 to C–10 and C–1' to C–6' based on standard nomenclature). Assignments of the carbon resonances were facilitated by *J*-modulated spin-echo experiments (*i.e.,* attached proton test) in which quaternary and secondary carbon nuclei cause negative singlets while tertiary and primary carbon nuclei result in positive singlets (Table 1). In the case of compound 1, eight negative and five positive singlets were observed. Based on symmetry, two carbon atoms of the B-ring were chemically equivalent; hence, only 13 signals from the 15-carbon molecule were expected and ultimately observed. Characterisation of the carbon resonances was also aided by information on 5,7-dihydroxyflavonoids reported by Breitmaier and Voelter [1987]. For example, the resonance of C–8 in these flavones appears in the range of 90 to 100 ppm. The ^{13}C chemical shift of the carbonyl resonance at C–4 generally appears in the range of 176 to 196 ppm depending on whether the compound is a flavone, isoflavone, dihydroflavone or flavonol.

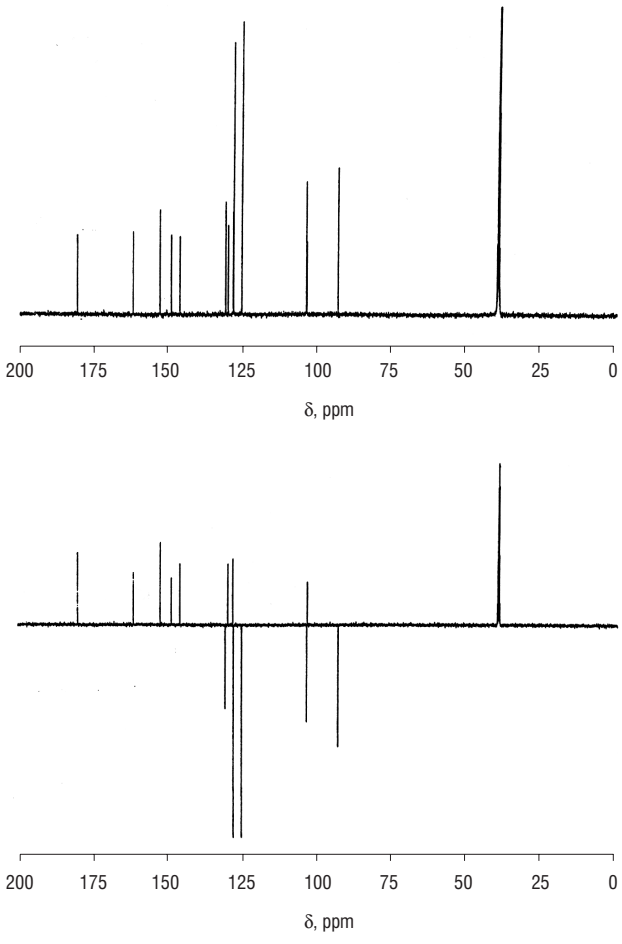

FIGURE 6. $^{13}C\{^{1}H\}$ NMR spectrum of compound 1.

TABLE 1. Assignment of $^{13}C\{^{1}H\}$ NMR signals (500 MHz, DMSO-d_6) for compound 1.

Carbon atoms[a]	*J*-modulated spin-echo	* (ppm)	Heteronuclear $^{1}H,^{13}C$ multiple-quantum coherence[a]	
			via ^{1}J(C,H)	via $^{2,3}J$(C,H)
Rings A + C				
2	-	163.8		H–C(2N/6N), H–C(3)
3	+	105.3	H–C(3)	H–C(3)
4	-	183.0		
5	-	147.8		H–C(8)
6	-	154.5		H–C(8)
7	-	150.7		
8	+	94.9	H–C(8)	
9	-	130.2		H–C(8)
10	-	105.1		H–C(3), H–C(8)
Ring B				
1N	-	131.8		H–C(3), H–C(3N/5N)
2N	+	127.2	H–C(2N)	H–C(6N), H–C(4N)
3N	+	130.0	H–C(3N)	H–C(5N)
4N	+	132.7	H–C(4N)	H–C(2N), H–C(6N)
5N	+	130.0	H–C(5N)	H–C(3N)
6N	+	127.2	H–C(6N)	H–C(2N), H–C(4N)

[a]Numbering of carbon atoms refers to the structure of compound 1 in Figure 1. [b]Assignments are based on HMQC (^{1}J) and HMBC ($^{2,3}J$) experiments.

Heteronuclear multiple quantum coherence experiments, optimised for ^{1}J(C,H) coupling, were run. Such experiments are employed to determine which ^{1}Hs of a molecule are bonded to which NMR-active heteronuclei (*e.g.*, ^{13}C or ^{15}N). This two-dimensional correlation is based on proton-detection, and therefore is quicker to run, involves shorter relaxation times and offers higher sensitivity when compared to conventional carbon-detected two-dimensional HETCOR experiments. The HMQC contour plot (Figure 7) revealed that five ^{1}H chemical shifts arising from seven protons of compound 1 were bonded directly to the ^{13}C signals at 94.9, 105.3, 127.2, 130.0 and 132.7 ppm (Table 1). The remaining eight carbon signals were either quaternary in nature or had hydroxyl groups attached to them.

Heteronuclear multiple bond coherence experiments were also performed. Such two-dimensional experiments are used to determine which 1Hs of a molecule are linked to which NMR-active heteronuclei (*e.g.*, ^{13}C or ^{15}N) over more than one bond (usually 2 or 3). In essence, the information obtained from the HMBC spectrum is an extension of that from the HMQC spectrum, but is more complicated to analyse. The pulse sequence of the spectrometer was optimised for ^{2}J(C,H) and ^{3}J(C,H) coupling constants and a filter was utilised to suppress correlations *via* ^{1}J(C,H). Unequivocal

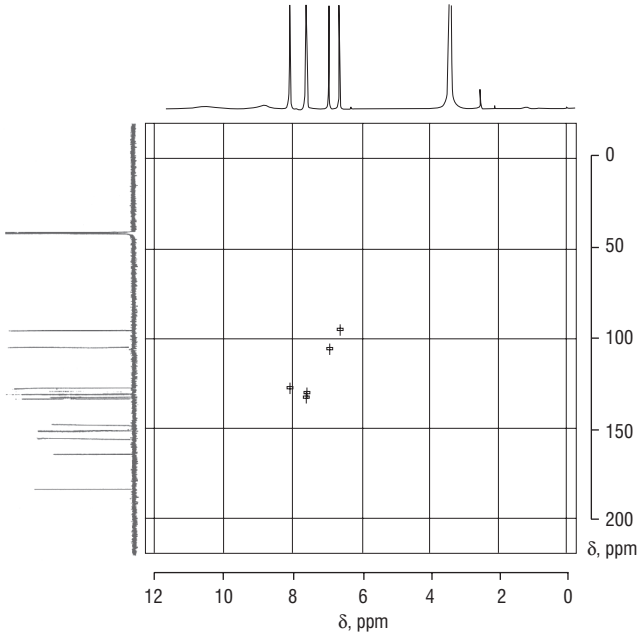

FIGURE 7. HMQC-NMR spectrum of compound 1.

assignments for the quaternary carbon atoms of compound 1 were achieved by means of the HMBC contour plot (Table 1). Although there is no way of knowing exactly how many bonds separate a H and C atom when a signal is observed, the most common correlations seen in HMBC spectra (Figure 8) involving aromatic rings are 3-bond correlations, because they are typically 7–8 Hz; this is the value for which the experiment is optimised. For example, in compound 1 the assignment of the quaternary carbon atom at C–10 was evident based on the observed ^{3}J(C,H) coupling between the ^{1}H singlets at both 6.93 ppm (bonded to C–3) and 6.64 ppm (bonded to C–8). In the case of C–2'/6', C–3'/5' and C–4' in ring B, the HMBC spectrum was somewhat complicated. The correlation for C–2'/6' indicated a possibility of ^{1}J(C,H) coupling. This is not uncommon as the suppression of ^{1}J(C,H) coupling does not work equally well for all proton signals. Despite the filter, such correlation signals may be seen in any HMBC spectrum. Although no ^{1}J(C,H) coupling was evident for the two protons in rings A and C, the aromatic nature of these rings places them in one plane, whereas the B-ring is angled away and exists in a different plane. On the other hand, the ^{1}H spectrum did indicate that the signals arising from the B--ring were behaving as a five-spin system. Consequently, the HMBC contour plot may actually be showing ^{3}J(C,H) coupling of C–2' with the 1H at C–6' and vice-versa. The HMBC contour plot also revealed ^{3}J(C,H) coupling for C–2'/6' with the ^{1}H at C–4', but no ^{2}J(C,H) coupling with the protons at C–3'/5'. This again is not surprising because, as stated above, spectra involving aromatic rings tend to show ^{3}J(C,H) coupling. This fact was also noted for C–3'/5' which showed either ^{1}J(C,H) coupling or ^{3}J(C,H) coupling of C–3' with the ^{1}H at C–5' and *vice-versa*. No ^{3}J(C,H) signal was observed at C–1', as this is a quaternary carbon atom. The signal at C–4' showed ^{3}J(C,H) coupling with the protons at C–2'/6'. To summarise, the NMR data obtained for compound 1 are consistent with the supposition that the compound is baicalein.

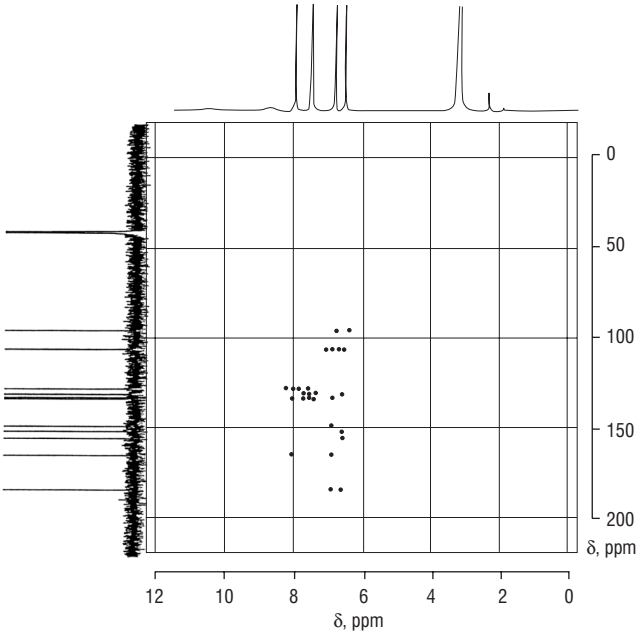

FIGURE 8. HMBC-NMR spectrum of compound 1.

Compound 2

Mass spectrometry. No EI mass spectrum could be acquired for compound 2; hence, the softer ionisation techniques of CI and FAB were employed. The FAB mass spectrum (Figure 9) showed a $[M + H]^+$ peak at an *m/z* of 447 (23.9%), and dominant peaks at *m/z* of 270 (15.4%) and 271 (14.1%) where the glucuronic acid moiety was cleaved from the glycoside. The CI mass spectrum (Figure 10), in which ammonia was used as the ionisation reagent, exhibited a $[M + H]^+$ peak at an *m/z* of 447 (20.5%), but no $[M + NH_4]^+$ signal at an *m/z* of 464. Mass spectra indicated that compound 2 has a molecular weight of 446 Da, which is characteristic of baicalin ($C_{21}H_{18}O_{11}$).

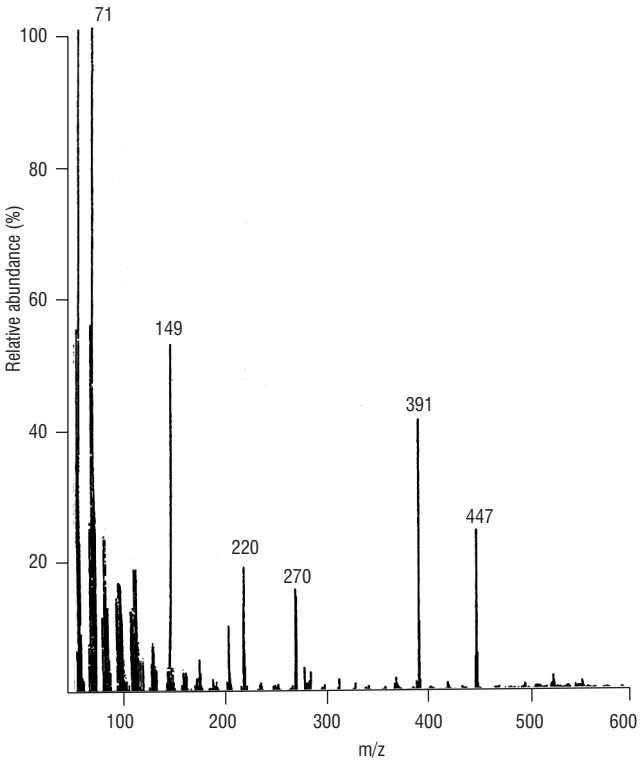

FIGURE 9. FAB-MS spectrum of compound 2.

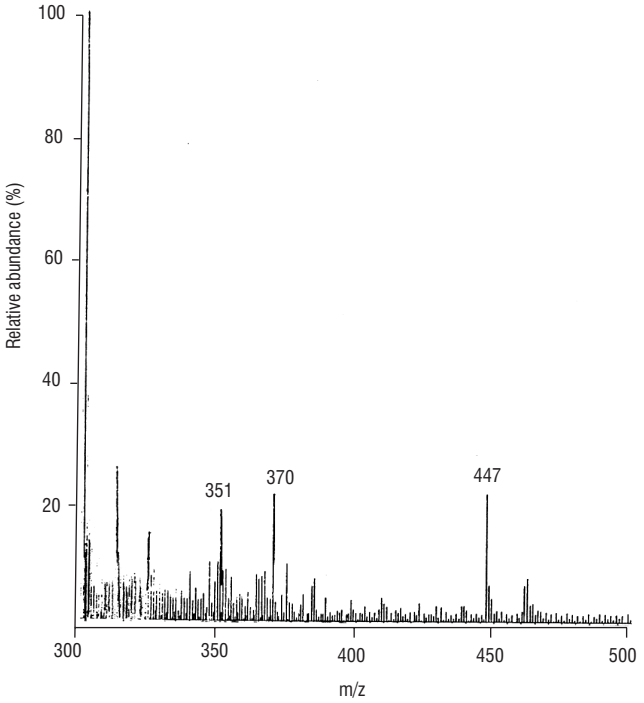

FIGURE 10. CI-MS spectrum of compound 2.

Nuclear Magnetic Resonance spectroscopy experiments. Based on chemical shifts (δ), integration and a deuterated water shake, the ^{1}H NMR spectrum of compound 2 (Figure 11) can be summarised as follows: δ (in ppm) ~3.4 [m, unconfirmed number of Hs, but most likely H–C(2''), H–C(3''), H–C(4'')], 4.07 [d, 1H, H–C(5'')], 5.25 [d, 1H, H–C(1'')], 7.00 [s, 1H, H–C(3)], 7.05 [s, 1H, H–C(8)], 7.60 [m, 1H, H–C(3')], 7.60 [m, 1H, H–C(5')], 7.60 [m, 1H, H–C(4')], 8.06 [dd, 1H, $^{3}J_{2'3'}$=7.52 Hz, $^{4}J_{2'4'}$=1.51 Hz, H–C(2')], 8.06 [dd, 1H, $^{3}J_{6'5'}$=8.09 Hz, $^{4}J_{6'4'}$=2.08 Hz, H–C(6')], 8.71 [s, 1H, ex, HO–C(6'')], 12.60 [s, 1H, ex, HO–C(5)]. There were a few broad signals with chemical shifts centred at 5.33, 5.53, 6.5 and 6.63 ppm; these might account for the hydroxyl groups of the glucuronic acid ring. According to Zhou *et al.* [1997], the coupling constant (J=7.5 Hz) of the anomeric proton signal at δ 4.25 indicates that the glucuronate moiety is in the β-configuration. Assignments were assisted from those made for compound 1.

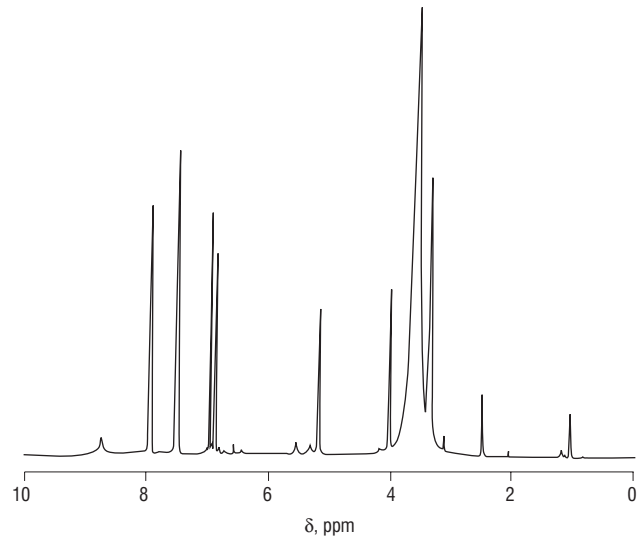

FIGURE 11. ^{1}H-NMR spectrum of compound 2.

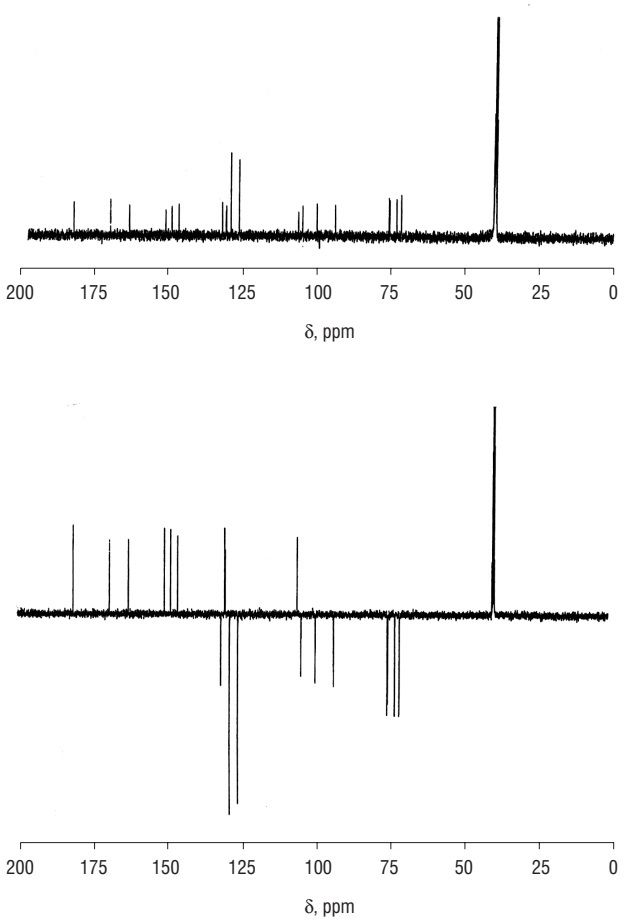

FIGURE 12. ^{13}C{^{1}H} NMR spectrum of compound 2.

As examples, the singlet at 7.00 ppm was the olefinic hydrogen atom in ring C and the singlet in ring A was evident at 7.05 ppm. The two sets of protons in the B-ring (*i.e.,* 2' & 6' and 3' & 5') behaved somewhat like a five-spin system. The doublet of doublets signal centred at 8.06 ppm is characteristic for the aromatic protons on the B-ring at 2' & 6', and as found in compound 1. The 1H signals of the aromatic protons at 3', 4' and 5' showed a multiplet, from which integration revealed that three protons were present at the signal centred at δ 7.60 ppm. As the fourth ring was a hexanoic acid and not a hexose, the proton signals at H–C(1'') and H–C(5'') have only one vicinal proton next to them. Hence, their signal appeared as doublets and assignments were easily made. The remaining three protons are adjacent to one another and are responsible for the multiplet centred at δ ~3.4 ppm.

The ^{13}C{^{1}H} NMR spectrum (Figure 12) showed 19 signals arising from one flavone (15 carbons, C–2 to C–10 and C–1' to C–6' based on standard nomenclature) and a hexanoic acid (δ 72.2, 73.7, 76.1, 76.4, 100.9 and 170.9 ppm), whose chemical shift values were in good agreement with those of β-D-glucuronic acid. Based on symmetry considerations, two carbon atoms of the B-ring were chemically equivalent; hence, only 13 signals from the 15-carbon molecule were observed. Instead of a hexose, a hexanoic acid was present: the strongly deshielded resonance from the carbon atom at δ 170 ppm is the result of an acid functional group. Characterisation of the carbon resonances was aided by the assignments made for compound 1 and by *J*-modulated spin-echo experiments (Table 2). For compound 2 there were nine negative and ten positive singlets. Information on

TABLE 2. Assignment of $^{13}C\{^1H\}$ NMR signals (500 MHz, DMSO-d_6) for compound 2.

Carbon atoms[a]	*J*-modulated spin-echo	* (ppm)	Heteronuclear $^1H,^{13}C$ multiple-quantum coherence[b]	
			via ^1J(C,H)	via $^{2,3}J$(C,H)
Rings A + C				
2	-	164.4		H–C(2N/6N), H–C(3)
3	+	105.6	H–C(3)	
4	-	183.4		H–C(3)
5	-	147.7		
6	-	150.1		H–C(8)
7	-	152.2		H–C(8), H–C(1O)
8	+	94.6	H–C(8)	
9	-	131.7		H–C(8)
10	-	106.9		H–C(3), H–C(8)
Ring B				
1N	-	131.5		H–C(3), H–C(3N/5N)
2N	+	127.2	H–C(2N)	H–C(6N), H–C(4N)
3N	+	130.0	H–C(3N)	H–C(5N)
4N	+	132.8	H–C(4N)	H–C(2N), H–C(6N)
5N	+	130.0	H–C(5N)	H–C(3N)
6N	+	127.2	H–C(6N)	H–C(2N), H–C(4N)
Glucuronic acid				
1O	+	100.9	H–C(1O)	H–C(3O), H–C(5O)
2O	+	73.7	H–C(2O)	H–C(4O)
3O	+	76.1	H–C(3O)	H–C(1O), H–C(5O)
4O	+	72.2	H–C(4O)	H–C(2O), H–C(5O)
5O	+	76.4	H–C(5O)	H–C(1O), H–C(3O)
6O	+	170.9		H–C(4O), H–C(5O)

[a]Numbering of carbon atoms refers to the structure of compound 1 in Figure 1. [b]Assignments are based on HMQC (^1J) and HMBC ($^{2,3}J$) experiments.

5,7-dihydroxyflavonoids reported by Breitmaier and Voelter [1987] was useful; these authors noted that glycosylation of a flavonoid hydroxy group shifts the phenolic carbon resonance 1–2 ppm upfield. This was evident at C–7.

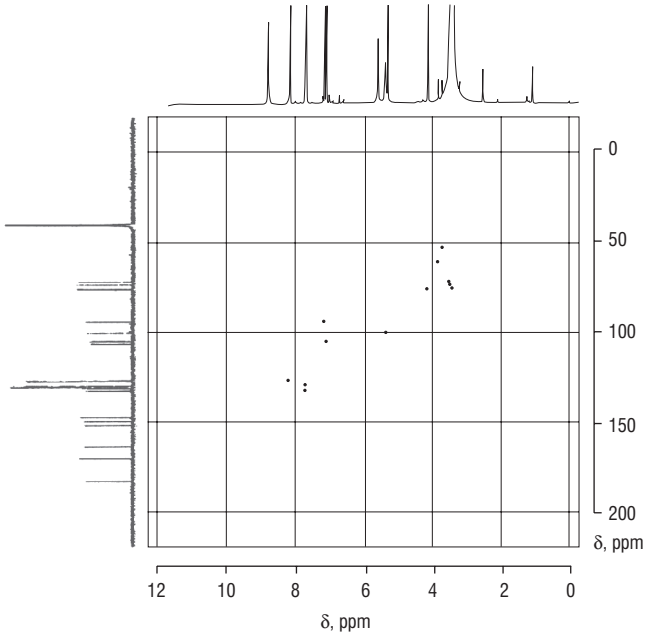

FIGURE 13. HMQC-NMR spectrum of compound 2.

Heteronuclear multiple quantum coherence experiments, optimised for ^1J(C,H) coupling, were run on compound 2. The HMQC contour plot (Figure 13) indicated that ^1J(C,H) coupling of 1Hs was occurring at ^{13}C signals of 72.2, 73.7, 76.1, 76.4, 94.6, 100.9, 105.6, 127.2, 130.0 and 132.9 ppm (see Table 2). Of particular note is that the doublet from the ^1H signal at 5.25 is bonded to the carbon atom at 100.9 and the doublet from the ^1H signal at 4.07 is bonded to the carbon atom at 76.4 ppm. These carbon signals must therefore be for C–1" and C–5" of the glucuronic acid moiety. The remaining nine carbon signals were either quaternary in nature or had hydroxyl groups attached to them.

Heteronuclear multiple bond coherence experiments gave more information. Unequivocal assignments for the quaternary carbon atoms of compound 2 were achieved by the HMBC contour plot (Figure 14) and comparison to assignments ascribed for compound 1 (Table 2). The position of attachment of the glucuronic acid in compound 2 was clear: the anomeric proton signal at δ 100.9 (H–1") showed long-range correlation with the carbon at 152.2 (C–7), thereby indicating that the glucuronate moiety is linked to the C–7 hydroxyl group of ring A. The observed ^3J(C,H) couplings from the ^1Hs of the glucuronic acid group with particular carbon atom signals as well as the HMQC data and assignments for the C–1" and C–5" positions allowed for complete characterisation of the carbon resonances at C–(2"), C–(3") and C–(4"). To summarise, the NMR data obtained for compound 2 are consistent with the supposition that the compound is baicalin.

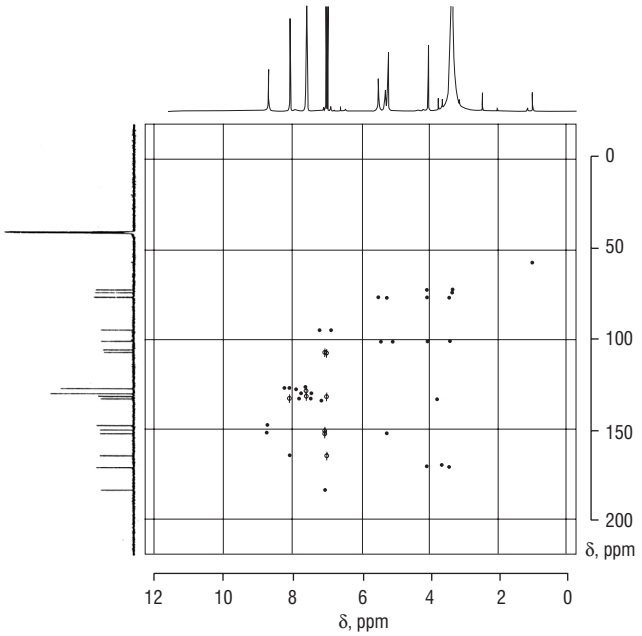

FIGURE 14. HMBC-NMR spectrum of compound 2.

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POTWIERDZENIE STRUKTURY CHEMICZNEJ ANTYOKSYDACYJNYCH TRIHYDROKSYFLAWONÓW Z *SCUTELLARIA BAICALENSIS*

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Z korzeni tarczycy bajkalskiej (*Scutellaria baicalensis*) związki fenolowe ekstrahowano metanolem. Z surowego ekstraktu 5,6,7-trihydroksyflawon (baikaleinę) i 5,6,7-trihydroksyflawon-7-*O*-β-D-glukopiranozydouronian (baikalinę) wyodrębniono za pomocą semipreparatywnej HPLC na kolumnie RP-18. Strukturę oczyszczonych trihydroksy-flawonów zbadano stosując spektometrię masową (EI-MS, CI-MS, FAB-MS) oraz NMR (^{1}H, $^{13}C\{^{1}H\}$, HMQC and HMBC-NMR).