Extraction of baicalin and characterization of its methylated derivatives. X-ray structure of 7-hydroxy-5,6-dimethoxyflavone 7-glucuronide methyl ester dihydrate

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Baicalin has been isolated and four of its methyl derivatives have been characterised by IR, UV, mass, ^{1}H and ^{13}C NMR and one of these, 7-hydroxy-5,6-dimethoxyflavone 7-glucuronide methyl ester, was subjected to an X-ray crystallographic analysis.

Introduction

Flavonoids are ubiquitous and their biological properties extend to antimicrobial, insecticidal and oestrogenic activities.[1] Baicilin (**1**) has been known for many years[2] and is found in Chinese medicinal preparations such as Sho-saiko-to[3] and Dang-Guei-San.[4] It has been evaluated as an inhibitor of HIV-1 production *in vitro*,[5] for anti-inflammatory activity[6] and analysed by HPLC[7,8] and second-derivative spectrophotometry.[9] Unequivocal structure assignment was made by X-ray crystallographic determination of the 7-hydroxy-5,6-dimethoxyflavone 7-glucuronide methyl ester dihydrate (**3**).

Experimental

Extraction of baicalin: Scutellaria amoena C.H. Wright was collected in Xichang, Sichuan, China and a specimen deposited in the Herbarium, Chengdu University of Traditional Chinese Medicine, China. Dried powdered root (1 kg) was extracted with water for 2 h at 80ºC and filtered. Acidifying the filtrate with dilute hydrochloric acid to pH 2 resulted in a precipitate, which was filtered and resuspended in fresh water. The acidity of this suspension was adjusted to pH 7.0 by addition of 40% NaOH. An equal volume of ethanol was added, and after 1 h at room temperature the mixture was filtered to remove insoluble material. The clear filtrate was acidified to pH 2 with dilute hydrochloric acid at 80ºC. The precipitate which formed on cooling was filtered, washed with water and dried under vacuum in a oven to obtained a yellow powder. Chromatography over silica (Merck), on elution with chloroform-methanol-water (65:45:12) gave compound **1** as a yellow crystalline powder (2.2 g).

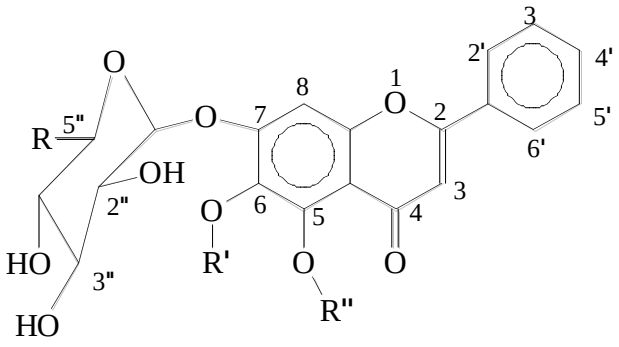
Compound **1** was characterised as baicalin by spectroscopic and chemical methods, in comparison with a reference sample (Central Drug Control Institute, State Public Health Administration, Beijing, China).

Baicalin (**1**): Melting point: 178–197ºC (dec) (Mettler FP50 melting point apparatus; uncorrected), IR (δ cm^{-1}) nujol: 3400, 1720,1660,1060 (Perkin-Elmer 681 spectrometer),

UV (λ_{max} nm) methanol: 277, 314 (Shimadzu UV-160A spectrometer), high resolution FABMS (*m/z*) Na^+ matrix: found 447.0940, required 447.0927 for $C_{21}H_{18}O_{11}$ $+H^+$,

^{1}H NMR (250 MHz, DMSO-d_6, δ ppm, TMS=0): [aglycone] 12.60 (C5-OH), 8.69 (C6-OH), 8.08 (H-2′ and H-6′), 7.61 (H-3′, H-4′ and H-5′), 7.05, 7.01 (H-3 and H-8), [glucuronyl] 5.52 (C2″-OH), 5.31(C3″-OH), 5.25 (H-1″), 4.05 (C4″-OH), other signals not assigned due to overlap. ^{13}C NMR (62.5 MHz, DMSO-d_6, δ ppm, TMS=0) were recorded.

Methylation: Baicalin was methylated with diazomethane to give a mixture of products. Chromatography over silica on elution with ethyl acetate:acetone (4:1) yielded *5,7-dihydroxy-6-methoxy flavone 7-glucuronide methyl ester* (**2**) and *7-hydroxy-5,6-dimethoxyflavone 7-glucuronide methyl ester* (**3**). The structure of compound (**3**) was unequivocally established by X-ray crystallography. The position of methylation in the mono-ether (**2**) was assigned by the comparison of chemical shifts in ^{1}H and ^{13}C NMR spectra with those exhibited by analogous glucoside-flavones (**4** and **5**).[10] In the ^{1}H NMR spectrum of (**1**) the 5- and 6-hydroxyl substituent protons resonate at δ 12.60 and 8.69 respectively, which is comparable to signals observed in the spectrum of glucoside (**4**). Methylation of these phenolic groups results in loss of the corresponding proton signals. Neither proton signal was observed in the spectrum of the dimethoxy compound (**3**). Compound (**5**), which showed only a signal at δ 8.87 due to the 6-OH, has been assigned as the 5-methoxy isomer.[10] In contrast, (**2**) showed



Flavone		R	R′	R″
1	Baicalin, [5,6,7-trihydroxyflavone 7-glucuronide]	COOH	H	H
2	5,7-dihdroxy-6-methoxyflavone 7-glucuronide methyl ester	$COOCH_3$	CH_3	H
3	7-hydroxy-5,6-dimethoxyflavone 7-glucuronide methyl ester	$COOCH_3$	CH_3	CH_3
4	5,6,7-trihydroxyflavone 7-glucoside	CH_2OH	H	H
5	6,7-dihydroxy-5-methoxyflavone 7-glucoside	CH_2OH	H	CH_3

* To receive any correspondence.

a signal at δ 12.83, suggesting that the 5-OH remained unsubstituted and hence this must be the 6-methoxy isomer. The presence of a uronic acid group at C'-6 in baicalin (**1**) resulted in a signal at δ 170.1, which contrasted with the ^{13}C spectrum for (**4**), which showed no sugar signal above δ100.8.

5,7-dihydroxy-6-methoxyflavone 7-glucuronide methyl ester (**2**): Melting point: 218–219°C (EtOAc/acetone), high resolution FABMS (*m/z*) Na+ matrix: found 475.1227 , required 475.1240 for $C_{23}H_{22}O_{11}$ $+H^+$, ^{1}H NMR (250 MHz, DMSO-d_6, δ ppm, TMS=0): 12.83 (C5-OH), 8.08-8.11 (H-2′ and H-6′), 7.61 (H-3′,H-4′ and H-5′), 7.13, 7.08 (H-3 and H-8), 5.64, 5.36, 5.53 (sugar hydroxyls at C2″ and C3″), 4.22 (C4″-OH), 3.77 (C6-OMe ether), 3.66 (C6″-OMe ester), other signals not assigned due to overlap.^{13}C NMR (62.5 MHz, DMSO-d_6, δ ppm, TMS=0) were recorded.

7-hydroxy-5,6-dimethoxyflavone 7-glucuronide methyl ester (**3**)**:** Melting point: 209–210ºC (methanol). High resolution FABMS (*m/z*) Na+ matrix: found 489.1387, required 489.1397 for $C_{24}H_{24}O_{11}$ $+H^+$. ^{1}H NMR (250 MHz, DMSO-d_6, δ ppm, TMS=0): 8.05 (H-2' and H-6′), 7.60 (H-3′,H-4′ and H-5′), 7.39, 6.83 (H-3 and H-8), 5.64, 5.37, 5.53 (sugar hydroxyls at C2″ and C3″), 4.24 (C4″-OH), 3.81 (C5-OMe ether), 3.80 (C6-OMe ether), 3.66 (C6″-OMe ester), other signals not assigned due to overlap. ^{13}C NMR (62.5 MHz, DMSO-d_6, δ ppm, TMS=0) were recorded.

X-ray crystallography

X-ray diffraction data were collected on an Delft Instruments FAST diffractometer and details of crystal and structure refinement are shown in Table 2.

The structure was solved using program SIR-92[11] and refined with program SHELXL-97.[12] The oxygen and carbon atoms were refined with anisotropic temperature factors and the hydrogen atoms were allowed to ride on their attached atoms with one of two common temperature factors (methyl or non-methyl). A molecular plot was obtained with program ORTEP3[13] and is shown in Fig. 1.(The crystallographic numbering scheme differs from the previous NMR numbering scheme.)

Simple flavones are non-chiral but the glucuronide ring introduces chirality into the structure of (**3**). Based on the known absolute configuration of Baicalin the chiral centres in (**3**) are: C18 (S), C19 (R), C20 (S), C21 (S) and C22 (S). The ten atoms of the fused benzopyran ring system are approximately planar and inclined at 8.7(3)° to the aryl ring. In 6-hydroxyflavone[14] this angle is 9.8(2)° but in ipriflavone[15] it is closer to 50.0°. The torsion angle C2 – C1 – C12 – C17 is 8.9(8)° and the C1 – C12 bond length here is 1.457(7) Å. The glucuronide methyl ester ring adopts a chair conformation with puckering parameters[16] calculated from PLATON[17] of θ = 4.0(6)° and ϕ = 61(8)°. The presence of -OH groups and two water molecules results in extensive hydrogen bonding (Table 8).

Table 2 Crystal data and structure refinement

Empirical formula	$C_{24}H_{24}O_{11}.2H_2O$
Formula weight	524.46
Temperature	150(2)K
Wavelength	0.71073Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 5.0466(15)Å
	b = 18.848(2)Å
	c = 24.621(11)Å
	α = 90°
	β = 90°
	γ = 90°
Volume	2341.9(12)Å^3
Z	4
Density (calculated)	1.488 Mg/m^3
Absorption coefficient	0.122 mm^{-1}
F(000)	1104
Crystal size	0.10 × 0.08 × 0.06mm
q range for data collection	1.98 to 25.12°
Index ranges	–5<=h<=5
	–22<=k<=21
	–28<=l<=19
Reflections collected	9377
Independent reflections	3630 [R(int) = 0.1156]
Observed reflections [I>2σ(I)]	1248
Refinement method	Full-matrix l.s. on F2
Number of parameters	355
Goodness-of-fit on F2 (S)	0.484
Final R indices [I>2σ(I)]	R1 = 0.0391, wR2 = 0.0586
R indices (all data)	R1 = 0.1243, wR2 = 0.0785
Final weighting scheme	calc w=1/[σ^2(Fo^2)]
Residual diffraction max.	0.216 e/Å3
Residual diffraction min.	–0.192 e/Å3

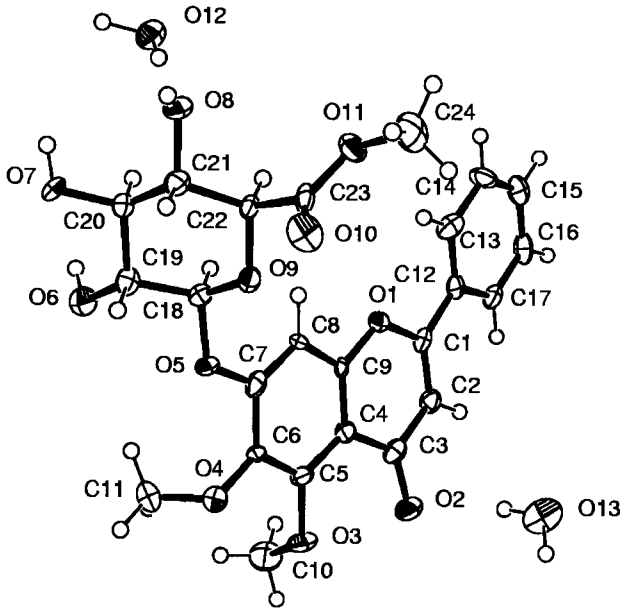


Fig. 1 The atomic arrangement in the molecule. Thermal ellipsoids are shown at the 50% probability level

We thank the EPSRC X-ray crystallographic service formerly at the University of Wales, Cardiff (now at Southampton University) for collecting the X-ray data and Dr K. Welham (School of Pharmacy, University of London) for mass spectral data.

Techniques used: IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry, X-ray crystallography.

References: 18

Schemes: 1

Fig.1 ORTEP plot of structure 3

Fig.2 Packing diagram of structure 3.

Table 1. ^{13}C NMR for structures 1,2,3,4, and 5.

Table 2. Crystal data and structure 3 refinement.

Table 3. Atom coordinates and U_{eq} for 3.

Table 4. Bond lengths and valency angles (non-H atoms) for 3.

Table 5. Torsion angles (non-H atoms) for 3.

Table 6. Anisotropic displacement parameters for 3.

Table 7. Hydrogen coordinates for 3.

Table 8. Hydrogen bonding in 3.

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Table 8 Hydrogen bonding in (3)

Type	D – H ... A	D... A (Å)	D – H (Å)	H...A (Å)	D – H ... A (°)
from H_2O	O12 – H1W...O2*	2.818(5)	1.02	2.16	120
from H_2O	O12 – H1W...O3*	3.073(6)	1.02	2.16	148
from H_2O	O12 – H2W...O8	2.957(5)	1.02	1.94	176
from H_2O	O13 – H3W...O7$^{\#}$	2.730(5)	1.01	1.81	150
from H_2O	O13 – H4W...O7	3.116(5)	0.99	2.48	121
from H_2O	O13 – H4W...O2*	3.186(6)	0.99	2.22	165
to H_2O	O6 – H6 ... O13	2.792(6)	0.84	2.02	153
inter	O7 – H7 ... O2*	2.677(5)	0.84	1.91	151
to H_2O	O8 – H8 ... O12^{+}	2.708(5)	0.84	1.91	157

Oxygen coordinates transposed by: * -?-x, 2-y, ?+z;
$^{\#}$ -1?-x, 2-y, -?+z;
$^{+}$ -1+x, y, z.

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