Front. Chem. China 2008, 3(2): 178–181
DOI 10.1007/s11458-008-0034-z

RESEARCH ARTICLE

Xiuhua REN, Guang DU, Bingfeng ZHOU, Kai ZONG, Baoxia MA

Study on spectroscopy of two flavonyl glycosides from *Andrographis paniculata*

Abstract To study the bioactive constituents from *Andrographis paniculata*, two compounds were isolated and purified by column chromatography on AB-8 macroporous adsorption resin and polyamide. Their structures were determined by various spectroscopic methods, including UV, FABMS, ^{1}H-NMR, ^{13}C-NMR, DQFCOSY, TOCSY, HMQC, HMBC, and NOESY. The ^{1}H-NMR and ^{13}C-NMR signals of the two compounds were assigned. The structures of the two compounds were elucidated as 5,4′-dihydroxy-7-methoxyflavone-6-yl-*β*-D-glucopyranoside and 5,4′-dihydroxy-7-methoxyflavone- 8-yl-*β*-D-glucopyranoside, respectively.

Keywords *Andrographis paniculata*, 5,4′-dihydroxy-7-methoxyflavone-6-yl-*β*-D-glucopyranoside, 5,4′-dihydroxy-7- methoxyflavone-8-yl-*β*-D-glucopyranoside, NMR, structure analysis

Andrographis panculata Nees is a small herb found throughout Southeast Asia and India. Extracts of this plant and its constituents mainly include diterpenoids and flavonoids and are reported to exhibit a wide spectrum of biological activities including antibacterial and anti-inflammatory properties [1]. Moreover, it has been reported to have some anti-thrombotic effect since the 1990s [2,3]. In the course of our anti-platelet aggregative active constituent studies, two compounds were isolated from the *Andrographis panculata Nees.* Then, these two compounds were identified by spectral analysis including ^{1}H-NMR, ^{13}C-NMR, DQFCOSY, TOCSY, HMQC, HMBC, and NOESY. Therefore, the ^{1}H-NMR and ^{13}C-NMR signals of these two compounds were assigned and the structures were elucidated as 5,4′-dihydroxy-7-methoxyflavon-6-yl-*β*-D-pyranoglucoside (**1**) and 5,4′-dihydroxy-7-methoxyflavon-8-yl-*β*-D-pyranoglucoside, respectively (**2**). Although the spectral properties of **1** including UV and IR were shown elsewhere [4–6], the ^{1}H-NMR and ^{13}C-NMR of **1** and **2** have not yet been reported. Thus, in order to provide more structural information about these compounds, we now report the ^{1}H-NMR and ^{13}C-NMR of **1** and **2**. Compound **1** was first isolated from *Andrographis panculata Nees*.

Translated from *Acta Chimica Sinica*, 2007, 65(14): 1399–1402 [译自: 化学学报]

Xiuhua REN (✉), Guang DU, Kai ZONG, Baoxia MA
Tongji Hospital of Tongji Medical College, Huazhong University of Science & Technology, Wuhan 430030, China
E-mail: renxiuhua_rxh@yahoo.com.cn

Bingfeng ZHOU
Hejiang Jingxin Pharmaceutical Co., Ltd., Xinchang, China

1 Experimental

1.1 General experimental procedures

All melting points were uncorrected and determined on an X4 melting point instrument. ^{1}H-NMR and ^{13}C-NMR and two-dimensional NMR spectra were measured on an INOVA-600 MHz Spectrometer from the Brucker Company, US. MS Spectra were measured on a Quattro Spectrometer. Column chromatographs were performed on AB-8 macroporous adsorption resin and polyamide from the Tiangji Nankai chemical company. TLC was carried out on silica gel plates from the Qingdao Ocean-chemical factory. All other regents were spectral or analytical grade.

1.2 Plant material

Andrographis panculata Nees were collected from Zhangpu in Fujian Province in August, 2002 and identified at the Fujian Institute for Drug Control.

1.3 Extraction and isolation

Dried *Andrographis panculata Nees* aerial parts (1 kg) were extracted three times from boiling water. The combined extracts were filtered and the filtrate was concentrated to obtain a syrup which was subjected to column chromatography on an AB-8 macroporous adsorption resin. After eluting with water and alcohol of different concentrations,

the elution was concentrated and identified by Thin-layer chromatographic analysis. The analogous fractions were collected and concentrated on a rotary evaporator under reduced pressure to obtain dried powders. The dried powders were dissolved in MeOH and subjected to column chromatography on polyamide with EtOH/H_2O as the eluent to give **1** (14 mg) and **2** (17 mg), respectively.

2 Results and discussion

2.1 5,4′-Dihydroxy-7-methoxyflavon-6-yl-β-D-glucopyranoside (**1**)

2.1.1 Physical and chemical properties, UV and MS spectral analysis

Compound **1** was obtained as canary powders, m.p. 208–211°C and showed a positive reaction in HCl-Mg and in Molish tests and an absorption maxima at 274 and 333 nm in the UV spectrum suggesting that it was a flavonyl glycoside. Furthermore, it gave a molecular ion at *m/z* 461.4 $[M–H]^-$ and peaks at 339.5, 299.3 [the parent flavoniod-H]$^-$, 283.5, 255.5, 113.3 by FABMS. This was consistent with the data reported in the literature [5] indicating that its molecular weight was 462.

2.1.2 NMR analysis

The ^{1}H-NMR spectrum of **1** showed the presence of phenyl-OH at $\delta_H = 13.52$ and resonances for an AA′BB′ system at $\delta_H = 8.42$ (dd, $J = 8.7$, 2.1 Hz, 2H), and $\delta_H = 7.28$ (dd, $J = 8.4$, 1.8 Hz, 2H), which were diagnostic for H-2′/6′ and H-3′/5′ of the B-ring. The resonance at $\delta_H = 6.88$ (d, $J = 1.2$ Hz, 1H) and $\delta_H = 6.65$ (d, $J = 1.2$ Hz, 1H) suggested that **1** had two substitutions in the A-ring. Additional signals at $\delta_H = 5.69$ (dd, $J = 7.5$, 1.5 Hz, 1H) and $\delta_H = 4.0–5.0$ (m, 6H) supported the presence of glucose in the β- pryranose form based on the coupling constant ($J = 7.5$ Hz). Also, a singlet at $\delta_H = 3.77$ was observed in the ^{1}H-NMR spectrum. The ^{13}C-NMR of **1** showed resonances for the parent flavonyl system at $\delta_C = 165.11$, 103.29, 183.04, 158.52, 126.43, 159.15, 96.52, 105.26, 122.30, 129.72, 116.91, and 162.96, and the resonances for the glucose system at $\delta_C = 105.97$, 76.03, 79.36, 71.64, 79.60, 62.76, respectively. The data for the glucose system further suggested that it was in the β- configuration in comparison with normal glucose data [7]. Also, a singlet at $\delta_C = 56.93$ was the $–OCH_3$ carbon signal. All the data of FABMS, ^{1}H-NMR and ^{13}C-NMR suggested that the formulas of **1** was $C_{22}H_{22}O_{11}$.

DQFCOSF is a double quantum filtered correlation spectroscopy which shows H-H coupling signals (^{1}J and ^{3}J), and establishs links to neighbor carbon atoms. The DQFCOSF spectrum of compound **1** show two doublets at $\delta = 8.42$ (H- 2′/6′) and $\delta = 7.28$ (H-3′/5′) further demonstrating a characteristic AA′BB′ system of a p-substituted phenyl ring and also showed the coupling signals between $\delta = 4.40$ (H-6″) and $\delta = 4.44$ (H-6″) (^{2}J), $\delta = 5.69$ (H-1″) and $\delta = 4.46$ (H-2″) (^{3}J), $\delta = 4.39$ (H-3″) and $\delta = 4.34$ (H-4″) (^{3}J), and $\delta = 4.34$ (H-4″) and $\delta = 4.02$ (H-5″) (^{3}J). This further demonstrated that the proton signals are characteristics of sugar. TOCSY, or total correlation spectroscopy, can be used to further verify the presence of AA′BB′ spin system of the B-ring and the glucose system. The TOCSY spectrum of compound **1** showed that there were coupling resonances between $\delta = 8.42$ (H-2′/6′) and $\delta = 7.28$ (H-3′/5′) which suggested that they were in a spin system. In addition, there were correlations between $\delta = 5.69$ (H-1″) and $\delta = 4.46$ (H-2″), $\delta = 4.39$ (H-3″) and $\delta = 4.34$ (H-4″) and $\delta = 4.34$ (H-4″) and $\delta = 4.02$ (H-5″), which further demonstrated that they were proton signals of glucose.

HMQC is ^{1}H-detected heteronuclear multiple-quantum coherence spectroscopy, which can establish the direct link to proton and carbon. It was used to identify the signals of carbon (Table 1). HMBC is ^{1}H-detected heteronuclear multiple bond correlated spectroscopy, which can correlate the proton and long-range carbon atoms. The HMBC spectrum of compound **1** showed that the resonances of $\delta = 6.88$ and $\delta_C = 165.11$ (C-2), $\delta_C = 105.26$ were correlated demonstrating $\delta_H = 6.88$ was the signal of H-3. The correlations between $\delta_H = 6.65$ and $\delta_C = 105.25$ (C-1″), 158.52 (C-9), 159.15 (C-7), and 126.43 (C-6) indicated it was the signal of H-8. In addition, the correlations between $\delta = 8.42$ (H-2′/6′) and $\delta_C = 165.11$ (C-2), $\delta = 5.69$ and $\delta_C = 126.43$ (C-6) and between $\delta = 3.71$ and $\delta_C = 159.15$ (C-7), indicated that the B-ring was linked to C-2, glucose was linked to C-6, and $–OCH_3$ was linked to C-7, respectively.

NOESY, namely nuclear Overhauser effect spectroscopy, exhibited intense NOE between $\delta = 13.53$ (5-OH) and both $\delta = 5.69$ (H-1″) and $\delta = 4.31$ (H-3″), $\delta = 8.42$ (H-2′/6′) and $\delta = 6.88$ (H-3), $\delta = 6.65$ (H-8) and $\delta = 3.77$ (H-3), $\delta = 5.19$ (H-1″) and $\delta = 4.02$ (H-5), further demonstrating that the glucose was linked to C-6, B ring was linked to C-2, $–OCH_3$ was linked to C-7 and glucose was in the β-pryranose form, respectively.

In all, using ^{1}H- and ^{13}C-NMR, HMQC, DQFCOSY, TOCSY, HMBC and NOESY spectroscopy, the compound **1** was identified as 5,4′-dihydroxy-7-methoxyflavon-6-yl-β-D-glucopyrano side. Its structure is shown in Fig. 1 and the data of ^{1}H-NMR, ^{13}C-NMR, HMBC and NOESY were displayed in Table 1.

2.2 5,4′- Dihydroxy-7-methoxyflavon-8-yl-β-D-glucopyranoside(**2**)

2.2.1 Physical and chemical properties, UV and MS spectral analysis

Compound **2** was obtained as canary powders, m.p. 228–230°C and shows positive reaction in HCl-Mg and Molish

Table 1 ^{1}H-NMR, ^{13}C-NMR,HMBC and NOESY of 5,4′-dihydroxy-7-methoxyflavon-6-yl-β-D-pyranoglucoside

carbon	δ_H	δ_C	HMBC (H→C)	NOESY (H→H)
2		165.11		
3	6.88 (d, J = 1.2 Hz)	103.29	C-3,C-2,C-10	H-2′/6′
4		183.04		
5		158.52		H-1″,H-3″
6		126.43		
7		159.15		
8	6.65 (d, J = 1.2 Hz)	96.52	C-8,C-10,C-9,C-7,C-6	7-OCH_3
9		158.52		
10		105.26		
1′		122.30		
2′/6′	8.42 (dd, J = 8.7, 1.2 Hz)	129.72	C-2,C-4′,C-2′/6′	H-3′/5′,H-3
3′/5′	7.28 (dd, J = 8.4, 1.8 Hz)	116.91	C-1′	H-2′/6′
4′		162.96		
1″	5.69 (dd, J = 7.5, 1.5 Hz)	105.97	C-1″,C-6	H-3″,H-5″
2″	4.459 ~ 4.461(m)	76.03	C-1″,C-2″	H-1″,H-4″
3″	4.391 ~ 4.395(m)	79.36	C-4″,C-3″	H-1″
4″	4.335 ~ 4.347(m)	71.64	C-6″,C-3″	H-2″
5″	4.02 (brt, J = 2.1 Hz)	79.60		
6″	4.422 ~ 4.438 (m)	62.76	C-5″	H-5″
	4.391 ~ 4.399 (m)		C-5″,C-6″,C-4″	H-6″,5-OH
7-OCH_3	3.77 (d, J = 1.2 Hz)	56.93	C-7	H-8

δ = 13.52(s) represented the signal of 5-OH

tests and absorption maxima at 270 and 330 nm in the UV spectrum suggesting that it was a flavonyl glycoside. Furthermore, it gave a molecular ion at m/z 461.4 [M–H]$^-$, and also the peaks at 321.4, 299.3 [the parent flavoniod-H]$^-$, indicating that its molecular weight was 462.

2.2.2 NMR analysis

The ^{1}H-NMR spectrum of **2** showed the presence of phenyl–OH at δ_H = 12.85 and 10.29, which disappeared after D_2O exchange and resonances for an AA′BB′ system at δ_H = 8.09 (d, J = 8.4 Hz, 2H), and δ_H = 6.92 (dd, J = 8.4, 2H), which were diagnostic for H-2′/6′ and H-3′/5′ of B-ring. The resonances at δ_H = 6.79 (s, 1H) and δ_H = 6.56 (s, 1H) were the proton signals of the parent flavones. Additional signals at δ_H = 4.81 (d, J = 7.2 Hz, 1H) and δ_H = 3.0–5.0 (m, 6H) supported the presence of glucose in the β-pyranose form. Also, a singlet at δ_H = 3.90 was observed in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum of **2** showed resonances for the parent flavonyl system at δ_C = 164.24, 102.30, 182.13, 158.41, 96.07, 156.94, 124.86, 149.10, 103.92, 121.29, 128.99, 115.84, and 161.25 and the resonances for the glucose system at δ_C = 103.62, 74.28, 76.39, 70.18, 77.17, and 61.18, respectively. The data of the glucose system further suggested that it was in β- configuration by comparison with the normal glucose data [7]. Further, a singlet at δ_C = 56.65 was –OCH_3 carbon signal. In all, based on the data of FABMS, ^{1}H-NMR, ^{13}C-NMR, it could be deduced that the formula of **2** was $C_{22}H_{22}O_{11}$.

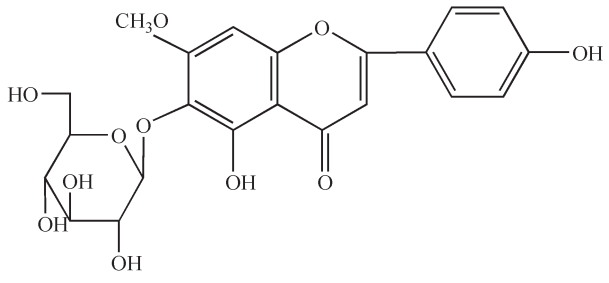

Fig. 1 Structure of 5,4′-dihydroxy-7-methoxyflavon-6-yl-β-D-pyranoglucoside

The structure analysis of compound **2** was the same as that of the compound **1**. The DQFCOSF spectrum of compound **2** showed two doublets at δ = 8.09 (H-2′/6′) and δ = 6.92 (H-3′/5′) indicating the presence of two –CH=CH– as a characteristic AA′BB′ system of a p-substituted phenyl ring. Also, the coupling signals between δ = 4.81 (H-1″) and δ = 3.36 (H-2″), δ = 3.27 (H-3″) and δ = 3.17 (H-4″), and δ = 3.17 (H-4″) and δ = 3.09 (H-5″) (^{3}J), suggested the presence of three –CHOH–CHOH–. The intense coupling signals between δ = 3.59 (H-6″) and δ = 3.36 (H-6″) (^{2}J) indicated the presence of one –CH2–, which was consistent with the proton signals of characteristic sugar. HMQC could establish the direct link to proton and carbon, and then it was used to identify the signals of carbon (Table 2). The HMBC spectrum of compound **2** showed the correlations between resonances δ = 6.79 and δ_C = 164.24 (C-2), δ_C = 182.13 (C-4) demonstrating that δ_H = 6.79 was the signal of H-3. Also, the correlations between δ_H = 6.56 and δ_C = 103.92 (C-10), 124.86 (C-8), 156.94 (C-7), and 158.41 (C-5) indicated that it was the signal of H-6. In addition, the correlations between δ = 8.09 (H-2′/6′) and δ_C = 164.24 (C-2), δ = 4.81 and δ_C = 124.86 (C-8), δ = 3.90 and δ_C = 96.07 (C-6), δ_C = 124.86 (C-8) and δ_C = 156.94 (C-7) demonstrated

Table 2 ^{1}H-NMR,^{13}C-NMR,DQFCOSY and HMBC of 5,4′-dihydroxy-7-methoxyflavon-6-yl-β-D-pyranoglucoside

carbon	δ_H	δ_C	DQFCOSY (H → H)	HMBC (H → C)
2		164.24		
3	6.79(s)	102.30		C-2,C-4,C-10
4		182.13		
5		158.41		
6	6.56(s)	96.07		C-10,C-8,C-5,C-7
7		156.94		
8		124.86		
9		149.10		
10		103.92		
1′		121.29		
2′/6′	8.09 (d, J = 8.4 Hz)	128.99	H-3′/5′	C-2,C-1′,C-4′
3′/5′	6.92 (d, J = 8.4 Hz)	115.84	H-2′/6′	C-1′,C-4′
4′		161.25		
1″	4.81 (d, J = 7.2 Hz)	103.62	H-2″	C-6″,C-5″,C-8
2″	3.36 (brt J = 7.8, 7.8 Hz)	74.28	H-3″	C-3″,C-1″
3″	3.246 ~ 3.292 (m)	76.39		C-4″
4″	3.17 (brt, J = 9.0, 8. 4 Hz)	70.18	H-5″	C-5″
5″	3.09 (brt, J = 6.6, 7.8 Hz)	77.17		C-4″,C-1″
6″	3.59 (d, J = 11.4 Hz)	61.18	H-5″,H-6″	C-5″,C-6″
	3.36 (d, J = 12.6 Hz)			
7-OCH_3	3.90 (s, 3 H)	56.65		C-6,C-8,C-7

that the B-ring was linked to C-2, glucose was linked to C-8, and –OCH_3 was linked to C-7, respectively.

In all, using ^{1}H and ^{13}C-NMR, HMQC, DQFCOSY and HMBC spectroscopy, the compound **2** was identified as 5, 4′-dihydroxy-7-methoxyflavon-8-yl-β-D-glucopyrano side. Its structure was shown in Figure 2, and the data of ^{1}HNMR, ^{13}CNMR, DQFCOSY and HMBC were displayed in Table 2. Compared to the spectroscopy data of the flavonoids II,VII [8], IV [9], VIII [10] reported in the literatures, the assignments of the ^{1}H-NMR and ^{13}C-NMR signals of the two compounds were reasonable.

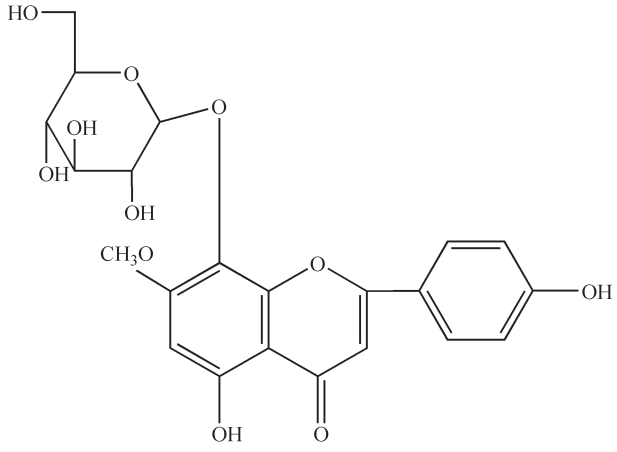

Fig. 2 Structure of 5,4′-dihydroxy-7-methoxyflavon-8-yl-β-D-pyranoglucoside

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