Determination of a new chromone from *Aurantii Fructus Immaturus* by DFT/GIAO method

Zhe Jiang\(^{ab}\), Fang Liu\(^{ab}\), Aijiao Zhong\(^{b}\), Jiaxiu Dugu\(^{b}\) and Xuezheng Li\(^{ab*}\)

\(^{a}\)Department of Pharmacy, Yanbian University Hospital, Yanji, Jilin Province 133000, P.R. China; \(^{b}\)Yanbian University College of Pharmacy, Yanji, Jilin Province 133000, P.R. China

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Investigation of EtOAc fraction from the 95% ethanol extract of *Aurantii Fructus Immaturus* led to the isolation of one new chromone (1) and seven known flavonoids (2–8). Their structures were elucidated mainly by NMR and HR-ESI-MS, as well as on comparison with the reported NMR data. The final substituent pattern of 1 was defined by comparison of \(^{13}\)C NMR data calculated by DFT/GIAO with those of experimental NMR data.

**Keywords:** *Aurantii Fructus Immaturus*; chromone; GIAO

1. Introduction

*Aurantii Fructus Immaturus* (Zhi Shi), being used as a traditional Chinese medicine, was the dried young fruit of *Citrus aurantium* L. or *Citrus sinensis* Osbeck. It is widely distributed in the provinces of Sichuan, Jiangxi, Fujian, Jiangsu, etc. Some of the chemical constituents are antioxidant, antiviral, antisepsis, anti-inflammatory, etc, in nature. It has been reported that *Aurantii Fructus Immaturus* had antitumour (Satoh et al. 1996) and protective activities on gastric mucosal lesions (Takase et al. 1994). It has also been shown to reduce the damage to the livers and kidneys in mice caused by the oral medication of *Magnolia officinalis* rehd (Chen et al. 2013), which aroused our interests in studying its chemical constituents. Thus, a systematic research on its chemical components was implemented and as a result, one new chromone, along with seven known flavonoids, was isolated. Herein, we report the isolation and structural elucidation of the new compound 1.
2. Results and discussion

One new chromone was isolated from the EtOAc fraction of the alcoholic extract of *Aurantii Fructus Immaturus* by HPLC along with seven known flavonoids, 5-demethylnobiletin (2) (Zhang et al. 2015), 5,6,7,8,4'-pentamethoxyflavone (3) (Chen et al. 1997), 5-hydroxy-6,7,3',4'-tetramethoxyflavone (4) (Peng et al. 2012), 5-hydroxy-6,7,8,4'-tetramethoxyflavone (5) (Chen et al. 2012), sinensetin (6) (Zhou et al. 2007), natsudaidai (7) (Yang et al. 2008) and 5,7,8,4'-tetramethoxyflavone (8) (Zhang et al. 2006). The structures of the isolated compounds were established based on 1D and 2D NMR spectral data. The substituent pattern of the aromatic ring was difficult to determine due to the similar $^{13}$C chemical shifts of different substituent patterns. The DFT/GIAO method (Borkowski et al. 2010) was adopted to compute the theoretical $^{13}$C NMR data with which the final substituent pattern of compound 1 was determined (Figure 1).

Compound 1 was obtained as a light yellow crystal with a molecular formula of $\text{C}_{10}\text{H}_8\text{O}_5$ as determined by data of the HRESI-MS ($m/z$ 209.0442 [M + H]$^+$). The $^1$H NMR spectrum of compound 1 showed six proton signals, including two phenolic hydroxyl proton signals at $\delta_{\text{H}}$ 10.85 (1H, s) and 12.39 (1H, s), two meta-coupled phenyl proton signals at $\delta_{\text{H}}$ 6.28 (1H, d, $J = 6.0$ Hz) and 8.27 (1H, d, $J = 6.0$ Hz), one methoxyl proton signal at $\delta_{\text{H}}$ 3.73 (3H, s) and one phenyl proton signal at 6.29 (1H, s). The $^{13}$C NMR spectrum gave nine sp$^2$ carbon signals at $\delta_{\text{C}}$ 99.6, 105.1, 110.8, 128.1, 150.4, 156.7, 157.6, 157.8 and 181.8, along with one methoxyl signal at $\delta_{\text{C}}$ 61.1. All the proton signals were assigned to their directly attached cartons by HSQC spectral data. As a result, NMR signals at $\delta_{\text{C}}$ 110.8($\delta_{\text{H}}$ 6.29), 157.7($\delta_{\text{H}}$ 8.27) and 181.8, being identical to those of 5,7-dihydroxychromone (Zhang et al. 2010), led to the elucidation of a $\alpha,\beta$-unsaturated pyrone ring. Compound 1 was also considered to be a chromone due to the resemblance of the NMR data of compound 1 and 5,7-dihydroxychromone.

There were two hydroxyl groups and one methoxyl group that were not assigned, according to the analysis described earlier. Being able to interact with the carbonyl group of C-4 to form a hydrogen bond, which enabled the proton chemical shift to be over twelve, the phenolic proton at $\delta_{\text{H}}$ 12.39 revealed the presence of a hydroxyl group at C-5. In the NOE spectrum, the correlation between –OH(C-5, $\delta_{\text{H}}$ 12.39) and H-6 ($\delta_{\text{H}}$ 6.29) was observed, leading to the determination of the location of the unassigned hydroxyl and methoxyl groups at C-7 and C-8. Thus, two possible structures of compound 1 can be derived as 1a and 1b as shown in Figure 2.

In order to reveal the final structure of compound 1, the DFT/GIAO method was adopted to calculate the $^{13}$C NMR data of 1a and 1b, respectively. The calculated and experimental carbon data were compared by a liner fit method. The $R$-square value of 1a was 0.998 (Figure 3), which

![Figure 1. Structures of compounds 1–8.](image)
was better than those of the 1b (0.993, Figure 3). Thus, the structure of 1 was established to be 5,7-dihydroxy-8-methoxychromone.

3. Experimental

3.1. General details
NMR analyses were measured on $^1$H NMR (400 MHz) and $^{13}$C NMR (100 MHz): Bruker DRX-400 spectrometer with TMS as internal standard. HR-ESI-MS was measured on a TOF of micromass spectrometer. Thin-layer chromatography was carried out on plates pre-coated with RP-18 gel (Merck, Darmstadt, Germany) and silica gel F$_{254}$ (Qingdao Marine Chemistry Ltd., Qingdao, China). Column chromatography (CC) was performed on silica gel (200–300 and 300–400 mesh; Qingdao Marine Chemical Factory, Qingdao, China), MPLC (BUCHI, column 3.5 cm × 45 cm, 50 μm) and HPLC (Shimadzu LC-8, column 10 mm × 250 mm, 5 μm).

3.2. Plant material
The dried young fruits of Citrus aurantium L. were purchased in An-guo medicine market, Hebei province, China. The plant was identified by Prof. Guanghai Shen of Yanbian University College of Pharmacy and a voucher specimen was deposited in the central experiment laboratory of Yanbian University Hospital (No. 20140123zh).

3.3. Extraction and purification of 1–8
Aurantii Fructus Immaturus (10 kg) was extracted three times by means of reflux with hot ethanol (30 L, 95% v/v) for 3 h each and the combined solution was concentrated in vacuo to
give syrup (1.25 L). Followed by suspension in water, the suspension was then extracted with petroleum ether, ethyl acetate and n-butanol, successively. The EtOAc crude extract (138 g) was applied on a silica gel column, eluted with petroleum ether – EtOAc gradient (from 100:1 to 0:1) to afford eight fractions. Fr.3 (petroleum ether – EtOAc 100:20, 8.6 g) was isolated by Sephadex LH-20 to give seven subfractions. The Fr. 3-7 was subjected to HPLC (MeOH–H₂O 75:25) to give compounds 2 (8.3 mg), 3 (6.5 mg) and 4 (9.8 mg). The Fr. 3-5 (petroleum ether – EtOAc 100:50, 20.5 g) was then subjected to semi-preparative reverse-phase HPLC (C-18) by eluting with MeOH–H₂O (65:35) to get compounds 5 (6.5 mg), 6 (8.0 mg), 7 (11.8 mg) and 8 (10.5 mg). The Fr. 3-7 (petroleum ether-acetone 100:70) gave compound 1 (7.5 mg) by repeated silica gel chromatography, Sephadex LH-20 and HPLC.

3.3.1. 5,7-Dihydroxy-8-methoxychromone (1)
Yellow crystal; HRESI-MS m/z 209.0442 [M + H]⁺ (calcd for C₁₀H₈O₅, 209.0450). UV λₘₐₓ 208, 226, 255; IR (KBr) νₘₐₓ 3422, 3091, 2859, 1662, 1424, 1279, 1164, and 1021 cm⁻¹. ¹H NMR (400 MHz, DMSO-d₆): 6 3.73, s, 3H, C₈-OMe; 6.28, d, J = 6.0 Hz, 1H, C₃-H; 6.29, s, 1H, C₆-H; 8.27, d, J = 6.0 Hz, 1H, C₂-H; 10.85, s, C₇-OH; 12.39, s, C₅-OH; ¹³C NMR (100 MHz, DMSO-d₆): δ (C-2) 157.6; (C-3) 110.8; (C-4) 181.8; (C-5) 156.7; (C-6) 99.6; (C-7) 157.8; (C-8) 128.1; (C-9) 150.4; (C-10) 105.1; (C-OMe) 61.1.

3.4. ¹³C NMR data calculation
The structures of 1a and 1b were optimised by the DFT method with b3lyp/6-311g(d). The optimised structures were subjected to calculation of ¹³C data by Gauss 09 program using the DFT/GIAO Method by b3lyp/6-31 + g (2d,p).

Disclosure statement
No potential conflict of interest was reported by the authors.

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Supplementary material
Supplementary material relating to this paper is available online http://dx.doi.org/10.1080/14786419.2015.1034713, alongside Figures S1–S4.

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