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Flavonoid glucuronides from *Picria fel-terrae*

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Abstract

Three flavonoid glucuronides are reported from a *n*-BuOH extract of *Picria fel-terrae* (Scrophulariaceae). The structures were established by UV, one- and two-dimensional NMR and mass spectrometry as apigenin 7-*O*-β-glucuronide, luteolin 7-*O*-β-glucuronide and apigenin 7-*O*-β-(2″-*O*-α-rhamnosyl)glucuronide, the latter one being a new compound.

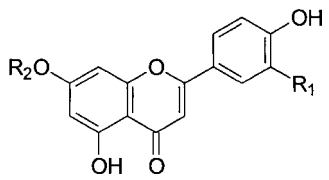
Keywords: Picria fel-terrae; Scrophulariaceae; Flavonoid glycosides; Flavonoid glucuronides; Apigenin 7-*O*-β-glucuronide; Luteolin 7-*O*-β-glucuronide; Apigenin 7-*O*-β-(2″-*O*-α-rhamnosyl)glucuronide

1. Introduction

In the southern part of China *Picria fel-terrae* Lour. (Scrophulariaceae) is used in traditional medicine against fever, herpes infections, cancer and inflammation. In a previous paper we have reported the isolation and characterisation of four complement inhibiting cucurbitacine glycosides from the *n*-BuOH fraction, obtained by partition of the aqueous extract (Huang et al., 1998). Here, we report the isolation and structure elucidation of three flavonoid glucuronides from the same plant.

2. Results and discussion

Three flavonoid glucuronides **1**–**3** were isolated by column chromatography on cellulose, polyamide, Sephadex LH-20 and silica gel from the *n*-BuOH fraction of *Picria fel-terrae*, obtained by partition of the aqueous extract and identified by UV, ^{1}H, ^{13}C and two- dimensional NMR spectroscopy and positive and negative FABMS. The UV spectra were typical of apigenin 7-glycosides (for **1** and **3**) and luteolin 7-*O*-glycosides (for **2**) (Markham, 1982). Compounds **1** and **2** were identified as apigenin 7-*O*-β-glucuronide and luteolin 7-*O*-β-glucuronide, respectively (Romussi, Fontana & De Tommasi, 1996).



	R_1	R_2
1	H	β-glucuronyl
2	H	β-glucuronyl
3	H	(2"-O-α-rhamnosyl)-β-glucuronyl

Compound **3** showed a molecular ion in positive FAB MS at m/z 593 $[M+H]^+$. The UV, ^{1}H and ^{13}C NMR spectra indicated the presence of an apigenin moiety and two sugar units. The position of attach-

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ment of the sugar moiety was determined by recording the UV spectra with the usual diagnostic reagents, indicating substitution at C-7 (Markham, 1982). The ^{13}C NMR spectrum was also in agreement with a 7-subtituted apigenin (Markham & Chari, 1982). The 12 remaining ^{13}C NMR signals were due to two hexose units. Two typical C-6 signals, a carbonyl at δ 171.48 and a methyl at δ 17.99 (corresponding in HSQC to a doublet at δ 1.18 (J = 6.2 Hz) in ^{1}H NMR) suggested a glucuronyl and a rhamnosyl moiety, respectively. This was in agreement with the presence of two anomeric protons in ^{1}H NMR, at δ 5.17 (d, J = 7.3 Hz), assigned to H-1″ of glucuronic acid and at δ 5.12 (d, J = 1.2 Hz), assigned to H-1‴ of rhamnose. The β-configuration of the glucuronic acid moiety was evident from the coupling constant of H-1″. The α-configuration of the rhamnosyl moiety was established by comparing its ^{13}C NMR assignments with published values for methyl β-L-rhamnoside and methyl α-L-rhamnoside (Agrawal, 1992), and by measuring the coupling constant $^{1}J_{CH}$ of the anomeric carbon, which was available from the residual coupling in the HMBC spectrum. $^{1}J_{CH}$ for C-1‴ was 173 Hz, which was in agreement with an α-configuration (Mizutani, Hayashi, Kasai & Tanaka, 1984), the difference between ^{1}J (C-1, H_{eq}) and ^{1}J (C-1, H_{ax}) being approximately 10 Hz in pyranoses (Hansen, 1981). The anomeric proton of rhamnose showed a long-range correlation in HMBC to a ^{13}C NMR signal at δ 76.18, which was directly correlated in HSQC to a ^{1}H NMR signal at δ 3.48. Because the multiplet at δ 3.48 was correlated in a ^{1}H-^{1}H COSY experiment to H-1″ of glucuronic acid at δ 5.17, it was concluded that glucuronic acid was substituted in position 2 with rhamnose. Detailed analysis of the ^{1}H-^{1}H COSY, HSQC and HMBC spectra allowed assignment of all ^{1}H and ^{13}C NMR signals of both the glycosyl and the apigenin moiety. To our knowledge, apigenin 7-*O*-β-(2″-*O*-α-rhamnosyl)glucuronide, or in general a (2″-*O*-α-rhamnosyl)glucuronide moiety, have not been reported previously. Apigenin 7-*O*-β-(2″-*O*-α-rhamnosyl)galacturonide was isolated before from *Silybum marianum* (Ahmed, Mabry & Matlin, 1989), but ^{13}C NMR assignments reported for this compound, especially for the uronic acid moiety, are different from those in this work, excluding the presence of a galacturonic acid unit in **3**. In addition, H-5 of glucuronic acid showed a typical doublet at δ 3.59 in ^{1}H NMR. The large coupling constant of H-5″ (d, J = 10.0 Hz) indicates an axial–axial relationship between H-4″ and H-5″, as expected in glucuronic acid, whereas in galacturonic acid the stereochemistry at C-4″ is reserved and an equatorial–axial relationship exists between H-4″ and H-5″. Based on these results, **3** was identified as apigenin 7-*O*-β-(2″-*O*-α-rhamnosyl)glucuronide.

3. Experimental

3.1. Plant material

The whole plant was collected in Lonlin, China in 1991 and identified by S.-Y. Liu, Department of Pharmaceutical Sciences, The Traditional Medicine College of Guangxi, China, where a voucher specimen is kept.

3.2. General

TLC was carried out on precoated silica gel 60 F_{254} plates (Merck), developed with EtOAC:HOAC:HCOOH:H_2O (30:0.8:1.2:8) (A). The Neu's spray reagent (1% diphenylboric acid ethanolamine complex in methanol) was used to visualise the spots. Column chromatography (CC) was carried out on cellulose, MN polyamide SC 6, Sephadex LH-20 and silica gel. ^{1}H, ^{13}C and 2D NMR spectra (including ^{1}H–^{1}H COSY, HSQC and HMBC) were recorded in CD_3OD and DMSO-d_6 on a Bruker DRX-400 instrument operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts are reported in ppm (δ). Fast atom bombardment (FAB) mass spectra were recorded in the positive and negative ion mode on a VG 70 SEQ instrument using glycerol as the liquid matrix. UV spectra were recorded on a UVIKON 931 spectrophotometer.

3.3. Extraction and isolation

Dried and powdered plant material (500 g, whole plant) of *Picria fel-terrae* was extracted exhaustively with warm water. The filtrate was concentrated under reduced pressure, then extracted with EtOAc and *n*-BuOH. The *n*-BuOH extract was evaporated under reduced pressure, and subjected to CC on cellulose (Avicel, 20–100 μm) eluted with a H_2O:MeOH gradient yielding 7 fractions. Fraction I was subjected to repeated CC on Sephadex LH-20 (Pharmacia, 25–100 μm) eluted with a Me_2CO:H_2O gradient, yielding compound **1** (58 mg). Fraction III was subjected to repeated CC on MN polyamide SC 6 (0.05–0.16 mm, Merck), eluted with a H_2O:EtOH gradient, giving 8 subfractions. Subfraction VI was subjected to repeated CC on Sephadex LH-20 using MeOH as eluent. This lead to the isolation of compound **2** (23 mg). Subfraction VII was subjected to repeated CC on silica gel. Elution with solvent EtOAC:HOAC:HCOOH:H_2O (30:1.2:0.8:8) yielded compound **3** (12 mg).

Acid hydrolysis of the glycosides and identification of the hydrolysis products was carried out as previously described (Cimanga et al., 1994). The sugars were identified by paper chromatography using a solvent system *n*-BuOH: pyridine:H_2O (6:3:1), by com-

parison with authentic samples (Merck) (detection with β-naphthol/H_2SO_4 reagent).

3.4. Apigenin 7-O-β-(2″-O-α-rhamnosyl)glucuronide (*3*)

R_f value, solvent system A, 0.10. UV λ_{max}^{MeOH} nm: 269, 334; $AlCl_3$, 276, 300, 346, 390; $AlCl_3$–HCl, 277, 300, 344, 390; NaOAC, 269, 336; NaOAc–H_3BO_3, 269, 334. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.81 (1H, s, H-3), 6.34 (1H, d, J = 1.8 Hz, H-6), 6.76 (1H, d, J = 1.8 Hz, H-8), 7.88 (2H, d, J = 8.8 Hz, H-2′, H-6′), 6.91 (2H, d, J = 8.8 Hz, H-3′, H-5′), 5.17 (1H, d, J = 7.3 Hz, H-1″), 3.48 (1H, m, H-2″), 3.45 (1H, m, H-3″), 3.19 (1H, m, H-4″), 3.59 (1H, d, J = 10.0 Hz, H-5″), 5.12 (1H, d, J = 1.2 Hz, H-1‴), 3.66 (1H, m, H-2‴), 3.31 (1H, dd, J = 9.4 Hz, J = 3.3 Hz, H-3‴), 3.19 (1H, m, H-4‴), 3.73 (1H, dd, J = 9.3 Hz, J = 6.2 Hz, H-5‴), 1.18 (3H, d, J = 6.2 Hz, H-6‴). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 164.23 (C-2), 102.91 (C-3), 181.84 (C-4), 160.90 (C-5), 99.37 (C-6), 162.61 (C-7), 94.31 (C-8), 156.92 (C-9), 105.29 (C-10), 120.54 (C-1′), 128.39 (C-2′, C-6′), 116.00 (C-3′, C-5′), 161.73 (C-4′), 97.56 (C-1″), 76.18 (C-2″), 77.44 (C-3″), 71.97 (C-4″), 73.30 (C-5″), 171.48 (C-6″), 100.41 (C-1‴), 70.32 (C-2‴ or C-3‴), 70.38 (C-2‴ or C-3‴), 71.80 (C-4‴), 68.22 (C-5‴), 17.99 (C-6‴). FABMS (positive ion mode) m/z: 593 $[M+H]^+$, 271.

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