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PATENSIN, A SAPONIN FROM *PULSATILLA PATENS* VAR. *MULTIFIDA*

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Key Word Index—*Pulsatilla patens* var. *multifida*; Ranunculaceae; triterpenoid glycoside; patensin.

Abstract—Patensin, a new triterpenoid glycoside, was isolated from the ethanolic extraction of the roots of *Pulsatilla patens* var. *multifida*. Its structure was established as hederagenin 3-*O*-*β*-D-galactopyranosyl-(1 → 2)-*β*-D-glucopyranoside on the basis of hydrolysis and spectral evidence including 1D and 2D NMR techniques.

INTRODUCTION

'Bai Tou Weng' has long been used as a traditional medicine against amoebae, vaginal trichromoniasis and bacteria in China [1]. Many species of the genus *Pulsatilla* have been used as 'Bai Tou Weng' [1]. Earlier, we reported the isolation and structure elucidation of several new triterpenoid glucosides from *Pulsatilla chinensis* [2–5]. In the present paper the isolation and structure elucidation of a new triterpenoid glycoside, patensin, are reported from the roots of *Pulsatilla patens* var. *multifida*. The structure of patensin has been shown to be hederagenin 3-*O*-*β*-D-galactopyranosyl-(1 → 2)-*β*-D-glucopyranoside (**3**) on the basis of hydrolysis and spectral evidence including ^{1}H–^{1}H COSY and ^{13}C–^{1}H NMR studies.

RESULTS AND DISCUSSION

Patensin (**3**), $C_{42}H_{68}O_{14}$, was obtained as a powder by ethanolic extraction of the roots of *Pulsatilla patens* var. *multifida* and subsequent partitioning with *n*-butanol–water followed by purification by chromatography using silica gel and Sephadex LH-20. Patensin on complete hydrolysis using methanol–hydrochloric acid afforded the known triterpene carboxylic acid hederagenin (**1**) [6], identified by infrared and TLC comparison and mixed melting point, as the aglycone and glucose and galactose as the sugars. The IR spectrum of patensin (**3**) exhibited hydroxyl adsorptions. The ^{1}H NMR spectrum of patensin showed 14 signals in the range δ3.60–5.60, attributable to the CH protons of the sugar residues. The ^{13}C NMR spectrum revealed the following signals: δ106.1 (CH), 104.2 (CH), 82.7 (CH), 78.2 (CH), 78.2 (CH), 76.7 (CH), 76.6 (CH), 75.6 (CH), 71.2 (CH), 69.9 (CH), 62.4 (CH_2), 62.2 (CH_2), again attributable to the sugar residues. The presence of two sugars in the molecule could be discerned from two signals for anomeric carbons at δ106.1 and 104.2. The ^{13}C NMR spectrum of patensin in pyridine-d_5 also showed signals at δ180.2 (–CO_2H), 144.9 (–C =) and 122.6 (= CH). All the ^{13}C NMR data of **3** are given in Table 1.

The hydroxyl groups at C-3 and C-23 and the carboxyl at C-17 in hederagenin, are available for glycosidic linkage with the sugar chains. In the ^{13}C NMR data of patensin (**3**), glycosylation shifts [7] were observed at C-2 (− 1.6 ppm) and C-3 (+ 9.2 ppm) by comparing with that of authentic hederagenin [6]. Thus the hydroxyl at C-3 was established as the position of the sugar linkage to the aglycone.

A complete assignment of the sugar protons was achieved through a study of the COSY NMR spectrum of **3**. In the ^{1}H–^{1}H COSY NMR, the well-separated anomeric hydrogens (δ 5.04, 1′ and δ5.30, 1″), the H-2′ (δ4.62) proton and the H-2″ (δ4.18) proton were assigned without difficulty. The vicinal coupling constants between 1′ and 2′ (7.5 Hz), and 1″ and 2″ (7.1 Hz) proved the configuration at both anomeric centres to be *β*. The chemical shifts of the sugar protons and their coupling constants are given in Table 2.

Partial hydrolysis of patensin (**3**) with 0.2 N HCl yielded a prosapogenin **2** which was isolated after purification by flash chromatography on silica gel. The ^{13}C NMR spectrum of **2** showed a signal at δ82.6 which indicated that the glucose group was attached at C-3 of aglycone. Therefore, **2** was identified as hederagenin 3-*O*-*β*-D-glucopyranoside [8]. Furthermore, in the long-range ^{1}H–^{1}H COSY spectrum of **3**, H-1′ of glucose (δ5.04) has a correlation to the H-3α of the aglycone (δ4.18). This evidence indicated that the inner sugar

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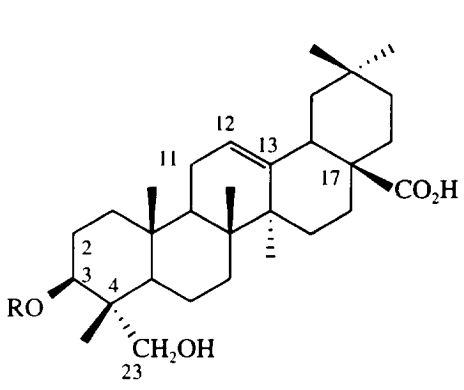

1 R = H
2 R = β-D-glucopyranosyl

3 R =

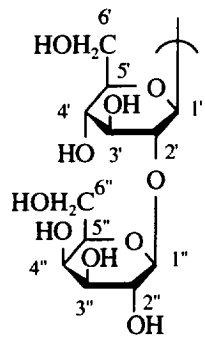

Table 1. ^{13}C NMR spectral data of **1**, **2** and **3** (δ, pyridine-d_5)

C	1*	2	3		C	1*	2	3
1	38.9	38.7	38.7		22	33.3	33.2	33.2
2	27.6	26.0	26.0		23	68.2	65.3	65.4
3	73.7	82.6	82.9		24	13.1	13.3	13.5
4	42.9	43.3	43.5		25	16.0	16.0	16.0
5	48.8	48.2	48.1		26	17.5	17.5	17.5
6	18.7	18.3	18.3		27	26.2	26.2	26.2
7	33.0	32.9	32.9		28	180.4	180.2	180.2
8	39.8	39.8	39.8		29	33.3	33.3	33.3
9	48.2	48.3	48.3		30	23.8	23.8	23.8
10	37.3	37.0	36.9	glc	1′		105.1	104.2
11	23.8	23.8	23.8		2′		75.5	82.7
12	122.7	122.6	122.6		3′		78.8	78.2
13	145.0	144.9	144.9		4′		71.9	71.2
14	42.2	42.2	42.2		5′		78.6	78.2
15	28.4	28.3	28.3		6′		63.0	62.4
16	23.8	23.7	23.7	gal	1″			106.1
17	46.7	46.2	46.6		2″			75.6
18	42.0	42.0	42.0		3″			76.6
19	46.5	46.4	46.4		4″			69.9
20	31.0	31.0	31.0		5″			76.7
21	34.3	34.2	34.2		6″			62.2

*Data taken from ref. 6.
glc: β-D-glucopyranosyl; gal: β-D-galactopyranosyl.

which was attached directly to the aglycone of **3** must be a glucose. By comparing the ^{13}C NMR spectrum of **2** with that of **3**, glycosylation shifts were observed at the signals of C-2′ (+ 7.2 ppm) and C-1′ (− 0.9 ppm) of the glucose group. Similarly, in the ^{13}C–^{1}H COSY spectrum of **3**, the carbon signal at δ82.7 was correlated to the proton signal at δ4.62 which was assigned as the H-2′ of the inner glucose by means of a ^{1}H–^{1}H COSY spectrum. These data disclosed that the C-2′ hydroxyl of the inner glucose must be linked to the terminal galactose.

Based on the above data, it is concluded that patensin has a disaccharide unit consisting of glucose and galactose at the C-3 position of hederagenin. Patensin is thus a new saponin and has been identified as hederagenin 3-*O*-β-D-galactopyranosyl-(1 → 2)-β-D-glucopyranoside (**3**).

EXPERIMENTAL

General. Mps: uncorr. Optical rotations were measured at 25°. IR spectra were recorded as KBr discs. The ^{1}H NMR spectra were run at 500 or 90 MHz, and the ^{13}C NMR spectra at 125 or 22.5 MHz with TMS as int. standard. The COSY NMR spectra were run using GE NMR spectrometer. The Electrospray mass spectrum was recorded on Fisons, VG Quattro. Chromatography was carried out using silica gel or Sephadex LH-20. TLC was carried out using silica GF_{254}. Vanillin–H_2SO_4 (saponin, sapogenin) and thymol–H_2SO_4 (sugars) were used as staining reagents.

Plant material. The roots of *Pulsatilla patens* var. *multifida* were collected at Chifun, in the Nei Monggol Autonomous Region, China, and authenticated by Dr

Table 2. Chemical shifts of sugar protons from the COSY NMR studies of **3** (pyridine-d_5, TMS)

Proton	(ppm)	Coupling constants (*J*, Hz)
glc	1′	5.04	*J* (1′, 2′) = 7.5
	2′	4.62	*J* (2′, 1′) = 7.5, *J* (2′, 3′) = 9.2
	3′	4.16	*J* (3′, 2′) = 9.2, *J* (3′, 4′) = 8.5
	4′	4.31	*J* (4′, 3′) = 8.5, *J* (4′, 5′) = 8.5
	5′	3.77†	
	6′a	4.38†	
	6′b	4.43	*J* (6′b, 6′a) = 13.5, *J* (6′b, 5′) = 4.0
gal	1″	5.30	*J* (1″, 2″) = 7.1
	2″	4.18	*J* (2″, 1″) = 7.1, *J* (2″, 3″) = 6.4
	3″	3.96	*J* (3″, 2″) = 6.4, *J* (3″, 4″) = 6.4
	4″	4.56	*J* (4″, 3″) = 6.4, *J* (4″, 5″) = 3.1
	5″	4.16†	
	6″a	4.38†	
	6″b	4.43	*J* (6″b, 6″a) = 13.5, *J* (6″b, 5″) = 4.0

†Obscured by other signals; therefore, coupling constants could not be determined accurately.

Xian-Min Cui. A voucher specimen has been deposited in the Herbarium of China Pharmaceutical University.

Extraction and isolation. Dried roots of *Pulsatilla patens* var. *multifida* (600 g) were refluxed with 95% EtOH (3 × for 2 hr each). The extracts were combined, evapd, dissolved in H_2O and defatted with $CHCl_3$ (H_2O–$CHCl_3$, 1:1). The defatted extract was partitioned twice with *n*-BuOH and the *n*-BuOH layer was evapd to dryness. The dark brown residue was dissolved in a small amount of MeOH and dropped into a 10-fold amount of Et_2O affording 50.5 g (8.40%) of a glycoside mixt. as a light brown powder. The mixt. (30 g) was subjected to Sephadex LH-20 (*ca* 200 g) chromatography using MeOH as eluant. Flash chromatography of the major saponin fr. on silica gel (*ca* 600 g) using $CHCl_3$–MeOH–H_2O (80:20:2) as eluant afforded **3**, mp 270–274°, $[\alpha]_D^{25}$ + 50.2° (MeOH; *c* 0.104). IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. ^1H NMR (pyridine-d_5): δ0.91 (^3H, *s*, Me), 0.94 (^3H, *s*, Me), 1.01 (^3H, *s*, Me), 1.02 (^3H, *s*, Me), 1.10 (^3H, *s*, Me), 1.25 (^3H, *s*, Me), 3.29 (^1H, *dd*, *J* = 13.9, 4.1 Hz), 4.42 and 3.77 (^2H, *m*, 23-H), 5.48 (^1H, *t*, C=CH). For sugar proton data see Table 2; ^{13}C NMR data are given in Table 1. ESMS (pyridine-d_5) *m/z*: 881.38 [M($C_{42}H_{68}O_{14}$) + C_5D_5N)]$^+$.

Partial hydrolysis of **3**. A soln of **3** (100 mg) in 0.2 N HCl (H_2O–dioxane, 1:1) (50 ml) was heated in a boiling water bath for 30 min. The reaction mixt. was neutralized with 1N NaOH, extracted with *n*-BuOH and passed through silica gel using $CHCl_3$–MeOH–H_2O (90:10:2) as eluant. The frs were evapd to dryness, dissolved in a small amount of MeOH and filtered through Sephadex LH-20 to give **2** (30 mg), mp 215–217°, IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. ^{13}C NMR data are given in Table 1.

Acid hydrolysis of **3**. Compound **3** (100 mg) was heated with 1.5 N HCl (H_2O–MeOH, 1:1) (60 ml) under reflux for 8 hr. The reaction mixt. was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was purified by Sephadex LH-20 with MeOH to afford prisms **1** (35 mg), *mp* 312–315°. Compound **1** was identified as hederagenin by direct comparison with an authentic sample (IR, TLC, mmp). The water layer of the hydrolysate was neutralized with Ag_2CO_3. The neutral hydrolysate revealed the presence of D-glucose and D-galactose by co-PPC with their authentic samples.

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