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A NOVEL KAEMPFEROL TRIGLYCOSIDE FROM FLOWER BUDS OF *Panax quinquefolium*

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Three kaempferol glycosides were isolated from the flower buds of Panax quinquefolium *L. together with kaempferol (**1**). By means of chemical and spectroscopic methods (NMR, ESI-MS, 2D NMR), their structures were established as trifolin (**2**), panasenoside (**3**), and kaempferol-3-*O-β-*D-glucosyl(1→2)-*β-*D-galactoside-7-*O-α-*L-rhamnoside (**4**).Compound **4** is a new compound.*

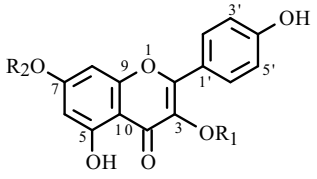
Key words: *Panax quinquefolium* L., kaempferol, flavone, panaxenoside.

Panax quinquefolium L. is a common medicinal material, but only its roots have been used since it was cultured successfully in China late in the 1970s. For chemical and commercial purposes, chemical research on the aerial part of *Panax quinquefolium* L. was started at the same time. Studies on the saponin and flavone contents in the leaves, stem, fruit, and flower buds have been reported partly [1–3]. Flavone compounds in the flower buds of *Panax quinquefolium* L. have been studied with the aim of providing a theoretical basis for the use of aerial parts of *Panax quinquefolium* L. In this paper, we describe the isolation and structure elucidation of four compounds **1**–**4** obtained from the flower buds of *Panax quinquefolium* L., including a new one **4**.

Compound **1** was isolated as yellow needles, soluble in warm alcohol. ^{1}H NMR (δ, ppm): 6.11 (1H, d, J = 2.0 Hz, H-6), 6.43 (1H, d, J = 2.0 Hz, H-8), 8.07 (2H, d, J = 10 Hz, H-2′,6′), 6.89 (2H, d, J = 10 Hz, H-3′,5′); ^{13}C NMR (δ, ppm):146.7 (C-2), 135.8 (C-3), 175.9 (C-4), 156.5 (C-5), 98.5 (C-6), 163.9 (C-7), 93.5 (C-8), 161.1 (C-9), 103.5 (C-10), 121.8 (C-1′), 129.6 (C-2′,6′), 115.5 (C-3′,5′), 159.6 (C-4′). The ^{1}H and ^{13}C NMR data of compound **1** corresponded to the data of kaempferol [2], so we identified compound **1** as kaempferol.

Compound **2** was isolated as yellow needles. ^{1}H and ^{13}C NMR assignments confirmed the aglycone as kaempferol. Acid hydrolysis of the compound afforded D-galactose as the residue, as confirmed by gas-liquid chromatography. The ^{1}H and ^{13}C NMR data of compound **2** (Table 1) corresponded to the data of trifolin [2], so we identified compound **2** as trifolin.

Compound **3** was isolated as yellow needles. ^{1}H and ^{13}C NMR assignments confirmed the aglycone as kaempferol. Acid hydrolysis of the compound afforded D-galactose and D-glucose as the residues as confirmed by gas-liquid chromatography. In the ^{13}C NMR, the signal due to C-2″ of galactose shifted downfield and appeared at δ 80.4 in comparison with kaempferol-3-galactoside. This is further supported by the HMBC correlation peak observed between the anomeric proton of glucose and a carbon at δ 80.4 assigned to C-2″ of galactose. Therefore, compound **3** was characterized as panasenoside.



1 - 4

1: $R_1 = R_2 = H$; **2:** $R_1 = \beta$-D-Gal*p*, $R_2 = H$
3: $R_1 = \beta$-D-Glc*p*-(1→2)-β-D-Gal*p*, $R_2 = H$
4: $R_1 = \beta$-D-Glc*p*-(1→2)-β-D-Gal*p*, $R_2 = \alpha$-L-Rha*p*

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TABLE 1. ^{1}H and ^{13}C NMR Data of Compounds **2–4** (DMSO-d_6, J/Hz)

C atom	**2**		**3**		**4**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	156.6		156.1		156.2	
3	133.6		132.7		133.3	
4	177.7		177.4		177.8	
5	161.1		161.1		161.1	
6	98.9	6.21 (1H, d, J = 2)	98.5	6.19 (1H, d, J = 2)	99.5	6.43 (1H, d, J = 2)
7	164.2		164.5		161.7	
8	93.9	6.43 (1H, d, J = 2)	93.5	6.43 (1H, d, J = 2)	94.5	6.83 (1H, d, J = 2)
9	156.6		155.4		156.1	
10	104.1		104.1		105.7	
1′	120.9		120.8		120.8	
2′6′	130.1	8.07 (2H, d, J = 9)	130.9	8.08 (2H, d, J = 9)	131.3	8.14 (2H, d, J = 9)
3′5′	115.5	6.88 (2H, d, J = 9)	115.1	6.89 (2H, d, J = 9)	115.4	6.92 (2H, d, J = 9)
4′	159.9		159.8		160.3	
Gal-1	101.9	5.25 (1H, d, J = 7)	98.1	5.68 (1H, d, J = 8)	98.5	5.69 (1H, d, J = 8)
2	71.4		80.4		80.6	
3	73.1		73.3		74.5	
4	68.2		67.4		67.7	
5	75.7		75.7		75.9	
6	60.3		59.8		60.1	
Glc-1			103.7	4.57 (1H, d, J = 8)	104.4	4.58 (1H, d, J = 8)
2			74.3		75.4	
3			76.9		77.1	
4			69.5		70.3	
5			76.5		76.7	
6			60.6		60.9	
Rha-1					98.5	5.34 (1H, d, J = 4)
2					72.1	
3					72.7	
4					72.9	
5					69.9	
6					18.1	

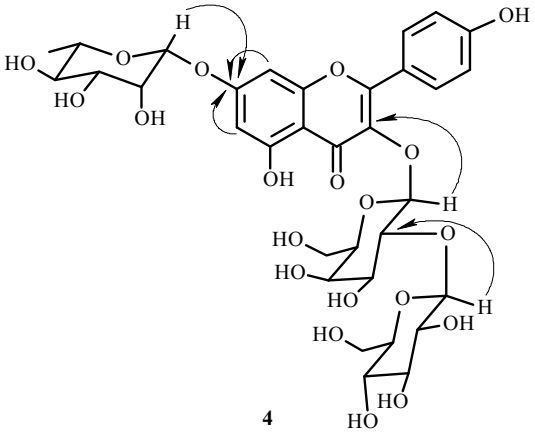

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Fig. 1. HMBC correlations of compound **4**.

Compound **4** was isolated as yellow needles, soluble in water, mp 224–226°C. The molecular formula $C_{33}O_{20}H_{40}$ was determined from high-resolution MS measurement and its negative and positive-mode ESI-MS [*m*/*z* 755.0 $(M–H)^–$, *m*/*z* 757.1 $(M+H)^+$, and *m*/*z* 779.2 $(M+Na)^+$)]. The ^{1}H and ^{13}C NMR assignments confirmed the aglycone as kaempferol. The acid

hydrolysis of compound afforded D-galactose, L-rhamnose, and D-glucose as the residues, as confirmed by gas-liquid chromatography [4]. In the ^{13}C NMR, the signal due to C-2″ of galactose shifted downfield and appeared at δ 80.6, in comparison with the ^{13}C NMR data of kaempferol-3-*O*-*β*-D-sophoroside-7-*O*-*α*-L-rhamnoside [5]. This is further supported by the HMBC correlation peak observed between the anomeric proton of glucose and a carbon at δ 80.6 assigned to C-2″ of galactose (Fig. 1). Also long-range correlation was observed between the following protons and carbons: H-1′ of the galactose and C-3, H-1‴ of rhamnose and C-7. Compound **4** is identified as kaempferol-3-*O*-*β*-D-glucosyl(1→2)-*β*-D-galactoside 7-*O*-*α*-L-rhamnoside.

EXPERIMENTAL

General Experimental Procedures. NMR spectra were recorded on Bruker-ARX-300 spectrometer, using TMS as an internal standard. ESI-MS was performed on a VG-5050E mass spectrometer. Gas chromatographic (GC) analysis was performed with a Fuli GC-9790 gas chromatograph equipped with H_2 flame ionization detector. The column was OV-17 on Silanox (0.3 mm × 50 m). Silica gel for chromatography was purchased from Qingdao Ocean Chemical Group Co. in China.

Plant Material. Flower buds of *Panax quinquefolium* L. were cultured in Jilin province, China and were identified by Prof. Qishi Sun of the Research Department of Natural Medicine, Shenyang Pharmaceutical University.

Extraction and Isolation. Dried flower buds (6 kg) of *Panax quinquefolium* L. were extracted with water and concentrated *in vacuo*. Then the extract was chromatographed on a macropore resin column with a step gradient of H_2O, 50% EtOH, and 80% EtOH. The 50% EtOH fraction was concentrated *in vacuo* and chromatographed on a silica gel column using gradient elution with a $CHCl_3$–MeOH mixture. Subfraction 11 [$CHCl_3$–MeOH (10:1), 300 mg] was re-chromatographed on a Sephadex-LH20 column eluted with MeOH to give compound **1** (15 mg). Subfraction 15 [$CHCl_3$–MeOH (100:15), 300 mg] was re-chromatographed on a Sephadex-LH20 column eluted with MeOH to give compound **2** (18 mg). Subfraction 11 [$CHCl_3$–MeOH (100:20), 300 mg] was re-chromatographed on a Sephadex-LH20 column eluted with MeOH to give compound **3** (25 mg) and compound **4** (25 mg).

Hydrolysis and GC Chromatography. Usually, 2 mg of the substance is incubated with 5 mL 1N HCl and refluxed for 45 min. The aqueous phase was extracted with EtOAc to separate the aglycones. The residue was taken to dryness using a rotary evaporator. The gas-liquid chromatography experiment was done using the published method [4].

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