

Novel acetylated flavonoid glycosides from the leaves of *Allium ursinum*

Hou Wu^a, Slavik Dushenkov^b, Chi-Tang Ho^{a,*}, Shengmin Sang^{c,d,*}

^a Department of Food Science, Rutgers University, 65 Dudley Road, New Brunswick, NJ 08901, United States

^b Rutgers University, Foran Hall, 59 Dudley Road, New Brunswick, NJ 08901, United States

^c Human nutrition research program, Julius L. Chambers Biomedical/Biotechnology Research Institute, North Carolina Central University, North Carolina Research Campus, 500 Laureate Way, Kannapolis, NC 28081, United States

^d Department of Chemistry, North Carolina Central University, 1801 Fayetteville Street, Durham, NC 27707, United States

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ABSTRACT

Seven flavonoid glycosides, kaempferol 3-*O*- α -L-rhamnopyranosyl (1 \rightarrow 2)-[3-*O*-acetyl]- β -D-glucopyranoside (**1**), kaempferol 3-*O*- α -L-rhamnopyranosyl (1 \rightarrow 2)-[6-*O*-acetyl]- β -D-glucopyranoside (**2**), kaempferol 3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside (**3**), kaempferol 3-*O*- β -D-glucopyranoside (**4**), kaempferol 3,7-di-*O*- β -D-glucopyranoside (**5**), 7-*O*- β -D-glucopyranosyl kaempferol 3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside (**6**), kaempferol 3-*O*- α -L-rhamnopyranosyl (1 \rightarrow 2)- β -D-glucopyranoside-7-*O*-[2-*O*-(*trans-p*-coumaroyl)]- β -D-glucopyranoside (**7**) were isolated from the *n*-butanol fraction of *Allium ursinum* L. and the structures of these compounds were elucidated on the basis of mass spectrometry, ¹H NMR, ¹³C NMR, HMQC and HMBC data. Among them, **1** and **2** are novel compounds and compounds **4** and **5** were isolated from this plant species for the first time.

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1. Introduction

Flavonoids are a group of polyphenolic compounds isolated from a wide range of plants (Pietta, 2000; Rice-Evan & Packer, 2003). Studies found that consumption of flavonoid-rich foods is associated with a lower incidence of heart disease, stroke, cancer, and other chronic diseases (Lotito & Frei, 2006). Many foods in human diets such as vegetables and fruits, contribute to the total daily intake of flavonoids in humans. *Allium* species are amongst the richest sources of dietary flavonoids and contribute to a large extent to the overall intake of flavonoids (Carotenuto et al., 1996; Carotenuto et al., 1997; Fattorusso, Lanzotti, Tagliatalata-Scafati, & Cicala, 2001; Slimestad, Fossen, & Vagen, 2007).

Allium ursinum L., which is also known as “ramson” and “wild garlic”, is a wild-growing *Allium* species in the forests of Europe and northern Asia (Schmitt, Schulz, Storsberg, & Keusgen, 2005). It is widely used as a spice as well as a traditional medicine. The leaves are edible and can be used as salad, spice, boiled as a vegetable, or as an ingredient for pesto in lieu of basil. The bulbs and flowers are also very tasty. It has been reported that wild garlic has a greater effect than regular garlic on lowering blood pressure of rats (Preuss, Cloutre, Mohamadi, & Jarrell, 2001). In addition, 1% wild garlic extract could significantly decrease total blood chole-

sterol level and increase HDL level (Preuss et al., 2001). A more recent study found that extract of the leaves of *A. ursinum* had strong antioxidant activity and this activity could be due to the high content of flavonoids (Stajner, Popovic, Canadanovic-Brunet, & Stajner, 2008). However, the chemical profile of the flavonoids in the leaves of *A. ursinum* has not been fully studied. Only five flavonoid glycosides have been reported from this plant (Carotenuto et al., 1996). In this paper, we described the isolation and structure elucidation of two novel acetylated flavonoid glycosides as well as five known flavonoid glycosides from this plant.

2. Material and Methods

2.1. Plant Materials

Plant material was provided by Lars Wilhjelm of Orenaes Estate, Falster, Denmark. *A. ursinum* L. was organically grown and harvested in March 2003. The fresh leaves were freeze dried shortly thereafter by Danish Freeze Dry (Kirke-Hyllinge, Denmark).

2.2. General procedure

¹H (600 MHz), ¹³C (150 MHz), and 2D NMR spectra were obtained on Varian AM-600 NMR spectrometers and with TMS as internal reference. ¹H-¹³C HMQC (heteronuclear multiple quantum correlation) and HMBC (heteronuclear multiple band correlation) experiments were performed as described previously (Fang et al., 2001). Negative ESI mass spectra were measured on a Finnigan

* Corresponding authors. Address: Human Nutrition Research Program, Julius L. Chambers Biomedical/Biotechnology Research Institute, North Carolina Central University, North Carolina Research Campus, 500 Laureate Way, Kannapolis, NC 28081, United States (S. Sang).

E-mail addresses: ho@aesop.rutgers.edu (C.-T. Ho), ssang@ncu.edu (S. Sang).

LTQ linear ion trap mass detector (ThermoFinnigan, San Jose, CA). Thin-layer chromatography was performed on Sigma–Aldrich TLC plates (250 μm thickness, 2–25 μm particle size), with compounds visualised by spraying with 5%(v/v) H_2SO_4 in ethanol solution.

2.3. Extraction and isolation

The dry leaves of *A. ursinum* L. (387 g) were extracted with 95% ethanol (4 l) at room temperature for three times. The extract was concentrated to dryness under reduced pressure, and the residue was suspended in water (500 ml) and partitioned successively with hexane (3×500 ml), ethyl acetate (3×500 ml) and *n*-butanol (3×500 ml). The *n*-butanol fraction was subjected to Diaion HP-20 column chromatography using an ethanol–water system (0–100%). The residue eluted by 70% aqueous ethanol was subjected to RP-C18 column chromatography eluted with 40% aqueous methanol to give compound **7** (105 mg) and 55% aqueous methanol to obtain **1** (30 mg), **2** (20 mg), **3** (25 mg), and **6** (100 mg). The residue eluted by 30% ethanol was subjected to Sephadex LH-20 column chromatography eluted with 90% aqueous ethanol to remove non-phenolic compounds and then applied to RP-C18 column chromatography eluted with 35% aqueous methanol to give compounds **4** (25 mg) and **5** (60 mg).

2.4. Spectrometric identification of isolated compounds

Kaempferol 3-*O*- α -L-rhamnopyranosyl (1 \rightarrow 2)-[3-*O*-acetyl]- β -D-glucopyranoside (**1**): yellow powder; negative ESI-MS, m/z 635 [M-H]⁻; ¹H and ¹³C NMR (CD_3OD): see Table 1.

Kaempferol 3-*O*- α -L-rhamnopyranosyl (1 \rightarrow 2)-[6-*O*-acetyl]- β -D-glucopyranoside (**2**): yellow powder; negative ESI-MS, m/z 635 [M-H]⁻; ¹H and ¹³C NMR (CD_3OD): see Table 1.

Table 1

¹H (600 MHz) and ¹³C (150MHz) NMR data for compounds 1 and 2 (CD_3OD) (δ in ppm, J in Hz).

Pos.	1		2	
	δH	δC	δH	δC
2		158.5 s		158.8 s
3		134.3 s		134.1 s
4		179.1 s		179.1 s
5		163.0 s		163.0 s
6	6.15 d (2.4)	99.7 d	6.15 brs	99.9 d
7		165.6 s		166.5 s
8	6.34 d (2.4)	94.6 d	6.35 brs	94.8 d
9		158.3 s		158.4 s
10		105.9 s		105.5 s
1'		123.0 s		123.1 s
2', 6'	8.02 d (9)	132.1 d	7.99 d (9)	132.0 d
3', 5'	6.89 d (9)	116.1 d	6.87 d (9)	115.9 d
4'		161.2 s		161.2 s
G				
1	5.82 d (7.2)	100.1 d	5.59 d (7.2)	100.2 d
2	3.72 m	79.0 d	3.60 m	79.8 d
3	5.13 t (9)	79.0 d	3.55 t (9)	78.7 d
4	3.54 m	70.2 d	3.31 m	71.5 d
5	3.38 m	78.0 d	3.37 m	75.3 d
6	3.74 m	62.2 t	4.20 brd (11.4)	63.8 t
	3.54 m		4.06 m	
Ac				
CO		172.3 s		172.3 s
CH3	2.16 s	21.1 q	1.75 s	20.3 q
R				
1	4.87 s	102.8 d	5.23 s	102.5 d
2	3.79 s	72.5 d	4.00 s	72.3 d
3	3.72 m	72.1 d	3.79 dd (8.4, 2)	72.2 d
4	3.30 m	73.8 d	3.34 m	74.0 d
5	4.00 m	69.6 d	4.07 m	69.8 d
6	0.97 d (6.6)	17.5 q	1.00 d (6.6)	17.5 q

Kaempferol 3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside (**3**): yellow powder; negative ESI-MS, m/z 593 [M-H]⁻. ¹H NMR (CD_3OD): δH 7.98 (2H, d, $J = 8.4$ Hz, H-2', 6'), 6.80 (2H, d, $J = 8.4$ Hz, H-3', 5'), 6.09 (1H, brs, H-8), 5.97 (1H, brs, H-6), 5.62 (1H, d, $J = 7.8$ Hz, H-G₁), 5.24 (1H, brs, H-R₁), 4.09 (1H, m, H-R₅), 4.0 (1H, m, H-R₂), 3.82 (1H, m, H-R₃), 3.70 (1H, brd, $J = 12$ Hz, H-G_{6a}), 3.64 (1H, t, $J = 9.0$ Hz, H-G₃), 3.53 (2H, m, H-G₂, G₄), 3.52 (1H, brd, $J = 12$ Hz, H-G_{6b}), 3.32 (1H, m, H-R₄), 3.19 (1H, brdd, $J = 9, 6$ Hz, H-G₅), 1.02 (3H, d, $J = 6.6$ Hz, H-R₆). ¹³C NMR (CD_3OD): δC 179.2 (C-4, s), 165.6 (C-7, s), 163.0 (C-5, s), 161.1 (C-4', s), 158.5 (C-9, s), 158.2 (C-2, s), 134.4 (C-3, s), 132.1 (C-2', 6', d), 123.1 (C-1', s), 116.1 (C-3', 5', d), 105.8 (C-10, s), 102.5 (C-R₁, d), 100.3 (C-G₁, d), 99.8 (C-6, d), 94.7 (C-8, d), 79.8 (C-G₂, d), 78.8 (C-G₃, d), 78.0 (C-G₅, d), 74.0 (C-R₄, d), 72.3 (C-R₂, d), 72.2 (C-R₃, d), 71.7 (C-G₄, d), 69.9 (C-R₅, d), 62.6 (C-G₆, t), 17.6 (C-R₆, q).

Kaempferol 3-*O*- β -D-glucopyranoside (**4**), yellow powder; negative ESI-MS, m/z 447 [M-H]⁻; ¹H NMR (CD_3OD): δH 8.04 (2H, d, $J = 9$ Hz, H-2', 6'), 6.87 (2H, d, $J = 9$ Hz, H-3', 5'), 6.39 (1H, d, $J = 1.8$ Hz, H-8), 6.19 (1H, d, $J = 1.8$ Hz, H-6), 5.23 (1H, d, $J = 7.8$ Hz, H-G₁), 3.69 (1H, dd, $J = 12, 1.8$ Hz, H-G_{6a}), 3.52 (1H, dd, $J = 9, 7.8$ Hz, H-G₂), 3.41 (2H, m, H-G₃, G_{6b}), 3.30 (1H, brdd, $J = 9, 5.4$ Hz, H-G₅), 3.20 (1H, t, $J = 9$ Hz, H-G₄). ¹³C NMR (CD_3OD): δC 179.4 (C-4, s), 165.9 (C-7, s), 162.9 (C-5, s), 161.5 (C-4', s), 159.1 (C-2, s), 158.5 (C-9, s), 135.1 (C-3, s), 133.2 (C-2', 6', d), 123.4 (C-1', s), 116.2 (C-3', 5', d), 106.1 (C-10, s), 104.0 (C-6, d), 100.4 (C-G₁, d), 95.2 (C-8, d), 78.4 (C-G₂, d), 78.0 (C-G₅, d), 75.7 (C-G₃, d), 71.3 (C-G₄, d), 62.5 (C-G₆, t).

Kaempferol- 3, 7-di-*O*- β -D-glucopyranoside (**5**): yellow powder; negative ESI-MS, m/z 609 [M-H]⁻; ¹H NMR ($\text{C}_5\text{D}_5\text{N}$): δH 8.37 (2H, d, $J = 9.0$ Hz, H-2', 6'), 7.14 (2H, d, $J = 9.0$ Hz, H-3', 5'), 6.97 (1H, d, $J = 2.4$ Hz, H-8), 6.75 (1H, d, $J = 2.4$ Hz, H-6), 6.36 (1H, d, $J = 7.2$ Hz, H-G₁), 5.07 (1H, m, H-G_{6a}), 4.57 (1H, m, H-G_{6b}), 4.56 (1H, m, H-G_{6a}), 4.42 (1H, m, H-G₅), 4.40 (1H, m, H-G_{6b}), 4.35 (1H, m, H-G₃), 4.33 (1H, m, H-G₂), 4.26 (1H, m, H-G₂), 4.25 (1H, m, H-G₃), 4.19 (2H, m, H-G₄, G₄), 4.04 (1H, m, H-G₅). ¹³C NMR ($\text{C}_5\text{D}_5\text{N}$): δC 177.5 (C-4, s), 162.6 (C-7, s), 160.9 (C-5, s), 160.5 (C-4', s), 156.3 (C-9, s), 155.5 (C-2, s), 130.5 (C-3, s), 129.5 (C-2', 6', d), 120.4 (C-1', s), 114.8 (C-3', 5', d), 105.5 (C-10, s), 102.1 (C-G₁, s), 100.3 (C-6, d), 99.0 (C-G₁, s), 94.7 (C-8, d), 77.9 (C-G₅, G₅, d), 77.2 (C-G₃, d), 77.1 (C-G₃, d), 74.7 (C-G₂, d), 73.4 (C-G₂, d), 70.1 (C-G₄, d), 69.7 (C-G₄, d), 61.2 (C-G₆, t), 61.0 (C-G₆, t).

7-*O*- β -D-glucopyranosyl kaempferol 3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside (**6**): yellow powder; negative ESI-MS, m/z 753 [M-H]⁻; ¹H NMR (CD_3OD): δH 8.05 (2H, d, $J = 9$ Hz, H-2', 6'), 6.78 (2H, d, $J = 9$ Hz, H-3', 5'), 6.72 (1H, brs, H-8), 6.44 (1H, d, $J = 1.8$ Hz, H-6), 5.68 (1H, d, $J = 7.8$ Hz, H-G₁), 5.24 (1H, brs, H-R₁), 5.05 (1H, d, $J = 7.2$ Hz, H-G₁), 4.07 (1H, m, H-R₅), 4.06 (1H, dd, $J = 8.4, 7.2$ Hz, H-G₂), 4.05 (1H, m, H-R₂), 3.92 (1H, brd, $J = 12$ Hz, H-G_{6a}), 3.84 (1H, dd, $J = 10.2, 3$ Hz, H-G_{6a}), 3.72 (1H, m, H-R_{6b}), 3.68 (1H, t, $J = 8.4$ Hz, H-G₃), 3.62 (1H, ddd, $J = 12, 8.4, 2.4$ Hz, H-G₅), 3.60 (1H, dd, $J = 9.6, 7.2$ Hz, H-G₂), 3.55 (1H, t, $J = 9.6$ Hz, H-G₃), 3.44 (1H, t, $J = 8.4$ Hz, H-G₄), 3.38 (1H, m, H-R₄), 3.37 (1H, brd, $J = 10.2$ Hz, H-G_{6b}), 3.34 (1H, t, $J = 9.6$ Hz, H-G₄), 3.29 (1H, m, H-G₅), 1.0 (1H, d, $J = 6$ Hz, H-R₆). ¹³C NMR (CD_3OD): δC 179.2 (C-4, s), 164.3 (C-5, 7, s), 162.8 (C-4', s), 159.5 (C-2, s), 157.8 (C-9, s), 134.3 (C-3, s), 132.2 (C-2', 6', d), 121.2 (C-1', s), 117.1 (C-3', 5', d), 107.6 (C-10, s), 102.5 (C-R₁, d), 101.5 (C-6, d), 100.6 (C-G₁, d), 100.3 (C-G₁, d), 95.7 (C-8, d), 79.9 (C-G₂, d), 78.8 (C-G₃, d), 78.2 (C-G₅, G₅, d), 77.7 (C-G₃, d), 74.6 (C-G₂, d), 74.0 (C-R₄, d), 72.3 (C-R₂, d), 72.2 (C-R₃, d), 71.8 (C-G₄, d), 71.2 (C-G₄, d), 69.9 (C-R₅, d), 62.6 (C-G₆, t), 62.4 (C-G₆, t), 17.6 (C-R₆, q).

Kaempferol 3-*O*- α -L-rhamnopyranosyl (1 \rightarrow 2)- β -D-glucopyranoside-7-*O*-[2-*O*-(*trans*-*p*-coumaroyl)]- β -D-glucopyranoside (**7**): yellow powder; negative ESI-MS, m/z 901 [M-H]⁻; ¹H NMR (CD_3OD): δH 8.04 (2H, d, $J = 9$ Hz, H-2', 6'), 7.67 (1H, d, $J = 15.6$ Hz, H-7''), 7.42 (2H, d, $J = 8.4$ Hz, H-2'', 6''), 6.87 (2H, d, $J = 9$ Hz, H-3',

5'), 6.76 (2H, d, $J = 8.4$ Hz, H-3'', 5''), 6.65 (1H, d, $J = 2.4$ Hz, H-8), 6.37 (1H, d, $J = 15.6$ Hz, H-8''), 6.35 (1H, d, $J = 2.4$ Hz, H-6), 5.72 (1H, d, $J = 7.2$ Hz, H-G₁), 5.32 (1H, d, $J = 7.8$ Hz, H-G'₁), 5.23 (1H, brs, H-R₁), 5.11 (1H, dd, $J = 9, 7.8$ Hz, H-G'₂), 4.02 (1H, m, H-R₅), 4.00 (1H, m, H-R₂), 3.95 (1H, dd, $J = 11.4, 1.8$ Hz, H-G'_{6a}), 3.82 (2H, m, H-R₃, G'_{6b}), 3.77 (1H, t, $J = 9$ Hz, H-G'₃), 3.71 (1H, dd, $J = 12.6, 2.4$ Hz, H-G'_{6a}), 3.62 (1H, brdd, $J = 11.4, 9$ Hz, H-G'₅), 3.61 (1H, dd, $J = 9.0, 7.2$ Hz, H-G₂), 3.54 (1H, t, $J = 9$ Hz, H-G₃), 3.53 (1H, t, $J = 9$ Hz, H-G'₄), 3.48 (1H, dd, $J = 12.6, 4.8$ Hz, H-G'_{6b}), 3.35 (1H, m, H-R₄), 3.27 (1H, H-4, t, $J = 9.0$ Hz, H-G₄), 3.23 (1H, ddd, $J = 9.0, 4.8, 2.4$ Hz, H-G₅), 0.95 (3H, d, $J = 6$ Hz, H-R₆). ¹³C NMR (CD₃OD): δ_c 179.0 (C-4, s), 168.0 (C-9'', s), 163.9 (C-7, s), 163.0 (C-5, s), 161.5 (C-4', s), 161.0 (C-4'', s), 160.6 (C-2, s), 157.5 (C-9, s), 147.3 (C-7'', d), 134.6 (C-3, s), 132.3 (C-2', 6', d), 131.2 (C-2'', 6'', d), 127.0 (C-1'', s), 122.7 (C-1', s), 116.8 (C-3'', 5'', d), 116.2 (C-3', 5', d), 114.8 (C-8'', d), 107.9 (C-10, s), 102.8 (C-R₁, d), 100.4 (C-G₁, d), 100.0 (C-6, d), 99.6 (C-R₁, d), 95.8 (C-8, d), 79.8 (C-G₂, d), 78.5 (C-G₃, d), 78.0 (C-G'₅, d), 77.8 (C-G₅, d), 75.5 (C-G'₃, d), 74.4 (C-G'₂, d), 73.4 (C-R₄, d), 71.9 (C-R₂, d), 71.8 (C-R₃, d), 71.5 (C-G₄, d), 71.0 (C-G'₄, d), 69.5 (C-R₅, d), 62.2 (C-G₆, t), 61.9 (C-G'₆, t), 17.5 (C-R₆, q).

3. Results and discussion

In our study, we found that all the flavonoids were in the *n*-butanol fraction. Therefore, the *n*-butanol fraction of *A. ursinum* L.

leaves was chromatographed successively on Diaion HP-20, Sephadex LH-20, and RP-C18 to afford 2 novel compounds (**1** and **2**) and five known compounds (**3–7**). The structures of compounds **3–7** were identified by comparison of their NMR and MS data with those reported in the literature (Carotenuto et al., 1996; Gall et al., 2003; Nakano, Murakami, Nohara, Tomimatsu, & Kawasaki, 1981). They are kaempferol 3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside (**3**), kaempferol 3-*O*- β -D-glucopyranoside (**4**), kaempferol 3, 7-di-*O*- β -D-glucopyranoside (**5**), 7-*O*- β -D-glucopyranosyl kaempferol 3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside (**6**), 7-*O*-[2-*O*-(*trans-p*-coumaroyl)]- β -D-glucopyranosyl kaempferol 3-*O*- α -L-rhamnopyranosyl (1 \rightarrow 2)- β -D-glucopyranoside (**7**). Among them, compounds **4** and **5** were isolated from this plant species for the first time. The structures of compounds **1–7** are shown in Fig. 1.

Compound **1**, a yellow powder, had a molecular formula of C₂₉H₃₂O₁₆ determined by negative-ion ESI-MS (at m/z 635 [M-H]⁻) as well as ¹³C NMR data. Its ¹H and ¹³C NMR spectra (Table 1) showed the signals for kaempferol. The ¹H NMR spectrum of **1** exhibited signals for A ring (H-6, 8 at δ_H 6.15 1H, d, $J = 2.4$ Hz and 6.34 1H, d, $J = 2.4$ Hz, respectively) and B ring (H-2', 6' and H-3', 5' at δ_H 8.02 2H, d, $J = 9.0$ Hz and 6.89 2H, d, $J = 9.0$ Hz, respectively) (Table 1). The ¹³C NMR spectrum of **1** showed signals for A ring (δ_c 163.0 C-5, 99.7 C-6, 165.6 C-7, 94.6 C-8, 158.3 C-9, and 105.9 C-10), B ring (δ_c 123.0 C-1', 132.1 C-2', 6', 116.1 C-3', 5', and 161.2 C-4'), and C ring (δ_c 158.5 C-2, 134.3 C-3, and 179.1 C-

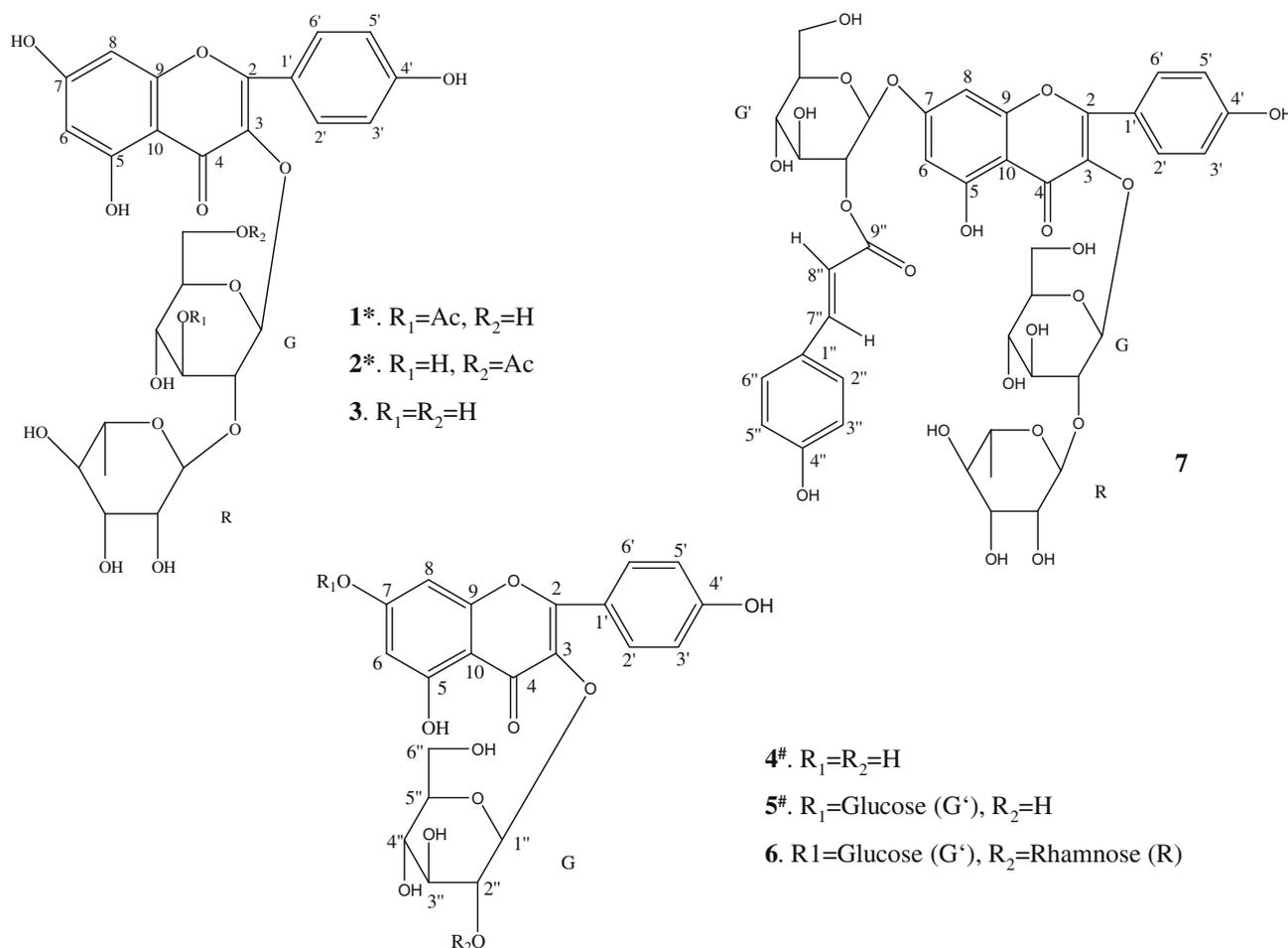


Fig. 1. Structures of compounds **1–7**, kaempferol 3-*O*- α -L-rhamnopyranosyl (1 \rightarrow 2)-[3-*O*-acetyl]- β -D-glucopyranoside (**1**), kaempferol 3-*O*- α -L-rhamnopyranosyl (1 \rightarrow 2)-[6-*O*-acetyl]- β -D-glucopyranoside (**2**), kaempferol 3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside (**3**), kaempferol 3-*O*- β -D-glucopyranoside (**4**), kaempferol 3,7-di-*O*- β -D-glucopyranoside (**5**), 7-*O*- β -D-glucopyranosyl kaempferol 3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside (**6**), kaempferol 3-*O*- α -L-rhamnopyranosyl (1 \rightarrow 2)- β -D-glucopyranoside-7-*O*-[2-*O*-(*trans-p*-coumaroyl)]- β -D-glucopyranoside (**7**), (* novel compounds and # compounds first isolated from *Allium ursinum*).

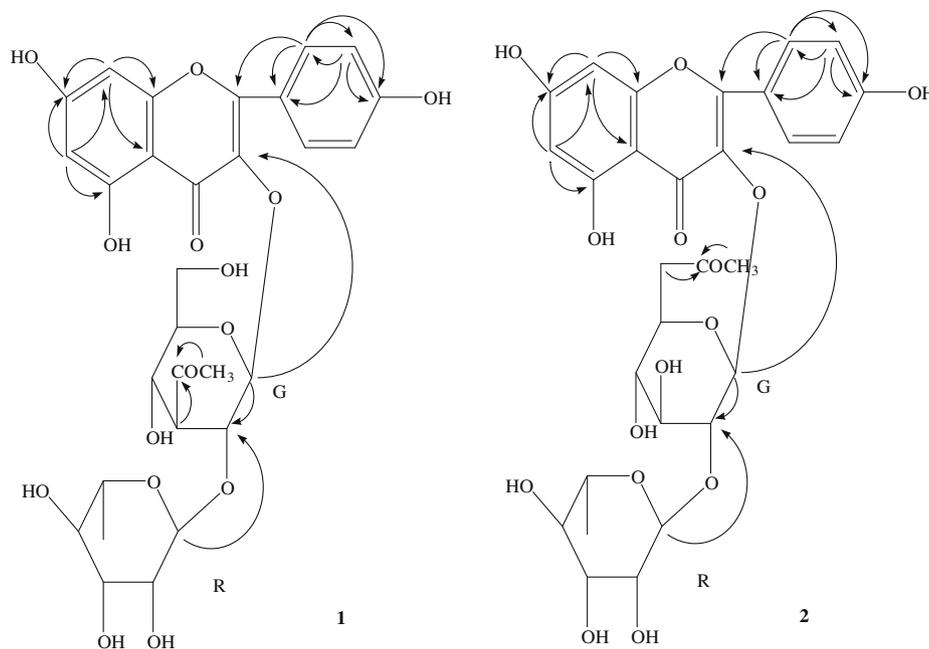


Fig. 2. Significant HMBC (H→C) correlations of compounds **1** and **2**.

4) (Table 1). Therefore, the aglycone of **1** was identified as kaempferol.

The identity of the sugars and the sequence of the oligosaccharide chain were determined by the analysis of a combination of its HMQC and HMBC NMR spectra. The β -anomeric configurations for the glucose unit were determined from its large $^3J_{H1, H2}$ coupling constant (7.2 Hz). The α -anomeric configuration for the rhamnose was determined by its chemical shifts at C-5 (δ_C 69.6). The HMBC spectrum showed cross peaks between C-3 (δ 134.3) and H-G₁ (δ_H 5.82), C-G₂ (δ 79.0) and H-R₁ (δ 4.87) (Fig. 2). Therefore, the glucose unit located at the C-3 position of kaempferol, and the rhamnose unit connected at the C-2 position of the glucose.

In comparison to the 1H and ^{13}C NMR spectra of the known compound **3**, compound **1** showed the signals for one acetyl group (δ_H 2.16, 3H, s and δ_C 21.1 q and 172.3 s). In addition, the molecular weight of **1** was 42 mass units higher than that of compound **3**. All these spectral features supported that compound **1** was the mono-acetylated derivative of compound **3**. The HMBC spectrum showed the cross peak between δ_C 172.3 and H-G₃ (δ_H 5.13, 1H, t, $J = 9.0$ Hz) indicating the acetyl group was located at the position C-3 of the glucose unit (Fig. 2). Thus, compound **1** was determined as kaempferol 3-O- α -L-rhamnopyranosyl (1→2)-[3-O-acetyl]- β -D-glucopyranoside.

The negative-ion ESI-MS of compound **2** displayed a molecular ion peak at m/z [M-H]⁻ 635, supporting a molecular formula of C₂₉H₃₂O₁₆, as noted above for compound **1**. The NMR spectra of **2** displayed signal patterns similar to those of **1** (Table 1). The 1H NMR spectrum of **2** showed signals for A ring (H-6, 8 at δ_H 6.15 1H, brs and 6.35 1H, brs, respectively) and B ring (H-2', 6' and H-3', 5' at δ_H 7.99 2H, d, $J = 9.0$ Hz and 6.87 2H, d, $J = 9.0$ Hz, respectively). The ^{13}C NMR spectrum of **2** showed signals for A ring (δ_C 163.0 C-5, 99.9 C-6, 166.5 C-7, 94.8 C-8, 158.4 C-9, and 105.5 C-10), B ring (δ_C 123.1 C-1', 132.0 C-2', 6', 115.9 C-3', 5', and 161.2 C-4'), and C ring (δ_C 158.8 C-2, 134.1 C-3, and 179.1 C-4). Therefore, the aglycone of **2** was also identified as kaempferol. It also had one acetyl group, a glucose unit, and one rhamnose unit. The HMBC spectrum of **2** showed cross peaks between C-3 (δ 134.1) and H-G₁ (δ 5.59), C-G₂ (δ 79.8) and H-R₁ (δ 5.23) (Fig. 2). Therefore, the

sequence of the oligosaccharide chain in compound **2** was the same as that in compound **1**. The major differences between **1** and **2** were the location of the acetyl group. In compound **2**, the acetyl group located at C-6 position of glucose unit, rather than the C-3 position in **1**. The HMBC spectrum of **2** showed correlations between δ_C 172.3 and H-G₆ (δ_H 4.20 brd 11.4 and 4.06 m) (Fig. 2). This confirmed that the acetyl group was located at C-6 position of glucose unit in **2**. Therefore, compound **2** was identified as kaempferol 3-O- α -L-rhamnopyranosyl(1→2)-[6-O-acetyl]- β -D-glucopyranoside.

References

- Carotenuto, A., Fattorusso, E., Lanzotti, V., Magno, S., Feo, V. D., & Circala, C. (1997). The flavonoids of *Allium neapolitanum*. *Phytochemistry*, 44, 949–957.
- Carotenuto, A., Feo, V. D., Fattorusso, E., Lanzotti, V., Magano, S., & Circala, C. (1996). The flavonoids of *Allium ursinum*. *Phytochemistry*, 41, 531–536.
- Fang, X., Qiu, F., Yan, B., Wang, H., Mort, A. J., & Stark, R. E. (2001). NMR studies of molecular structure in fruit cuticle polyesters. *Phytochemistry*, 57, 1035–1042.
- Fattorusso, E., Lanzotti, V., Tagliatalata-Scafati, O., & Cicala, C. (2001). The flavonoids of leek, *Allium porrum*. *Phytochemistry*, 57, 565–569.
- Gall, G. L., Dupont, M. S., Mellon, F. A., Davis, L. A., Collins, G. J., Verhoeyen, M. V., et al. (2003). Characterization and content of flavonoid glycosides in genetically modified tomato (*Lycopersicon esculentum*) fruit. *Journal of Agricultural and Food Chemistry*, 51, 2438–2446.
- Lotito, S. B., & Frei, B. (2006). Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? *Free Radical Biology Medicine*, 41, 1727–1746.
- Nakano, N., Murakami, K., Nohara, T., Tomimatsu, T., & Kawasaki, T. (1981). The constituents of Paris verticillata M. V. Bibe. *Chemical and Pharmaceutical Bulletin*, 29, 1445–1451.
- Pietta, P. G. (2000). Flavonoids as antioxidants. *Journal of Natural Products*, 63, 1035–1042.
- Preuss, H. G., Cloutre, D., Mohamadi, A., & Jarrell, S. T. (2001). Wild garlic has a greater effect than regular garlic on blood pressure and blood chemistries of rats. *International Urology and Nephrology*, 32, 525–530.
- Rice-Evan, C. A., & Packer, L. (2003). *Flavonoids in Health and Disease*. New York: Marcel Dekker Inc.
- Schmitt, B., Schulz, H., Storsberg, J., & Keusgen, M. (2005). Chemical characterization of *Allium ursinum* L. Depending on harvesting time. *Journal of Agricultural and Food Chemistry*, 53, 7288–7294.
- Slimestad, R., Fossen, T., & Vagen, I. M. (2007). Onions: a source of unique dietary flavonoids. *Journal of Agricultural and Food Chemistry*, 55, 10067–10080.
- Stajner, D., Popovic, B. M., Canadanovic-Brunet, J., & Stajner, M. (2008). Antioxidant and scavenger activities of *Allium ursinum*. *Fitoterapia*, 79, 303–305.