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

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ABSTRACT

Seven flavonoid glycosides, kaempferol 3-*O*-α-L-rhamnopyranosyl (1→2)-[3-*O*-acetyl]-β-D-glucopyranoside (**1**), kaempferol 3-*O*-α-L-rhamnopyronosyl (1→2)-[6-*O*-acetyl]-β-D-glucopyranoside (**2**), kaempferol 3-*O*-α-L-rhamnopyranosyl-(1→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→2)-β-D-glucopyranoside (**6**), kaempferol 3-*O*-α-L-rhamnopyranosyl (1→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, ^{1}H NMR, ^{13}C 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.

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, Taglialatela-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, Clouatre, Mohamadi, & Jarrell, 2001). In addition, 1% wild garlic extract could significantly decrease total blood cholesterol 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 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 glucosides 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 glucosides 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

^{1}H (600 MHz), ^{13}C (150 MHz), and 2D NMR spectra were obtained on Varian AM-600 NMR spectrometers and with TMS as internal reference. ^{1}H–^{13}C 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

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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→2)-[3-*O*-acetyl]-β-D-glucopyranoside (**1**): yellow powder; negative ESI-MS, m/z 635 $[M-H]^-$; ^1H and ^{13}C NMR (CD_3OD): see Table 1.

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

Table 1
1H (600 MHz) and 13C (150MHz) NMR data for compounds 1 and 2 (CD3OD) (δ in ppm, J in Hz).

	1		2	
Pos.	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→2)-β-D-glucopyranoside (**3**): yellow powder; negative ESI-MS, *m/z* 593 $[M-H]^-$. ^1H NMR (CD_3OD): δ_H 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-G_1), 5.24 (1H, brs, H-R_1), 4.09 (1H, m, H-R_5), 4.0 (1H, m, H-R_2), 3.82 (1H, m, H-R_3), 3.70 (1H, brd, *J* = 12 Hz, H-G_{6a}), 3.64 (1H, t, *J* = 9.0 Hz, H-G_3), 3.53 (2H, m, H-G_2, G_4), 3.52 (1H, brd, *J* = 12 Hz, H-G_{6b}), 3.32 (1H, m, H-R_4), 3.19 (1H, brdd, *J* = 9, 6 Hz, H-G_5), 1.02 (3H, d, *J* = 6.6 Hz, H-R_6). ^{13}C NMR (CD_3OD): δ_C 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-R_1, d), 100.3 (C-G_1, d), 99.8 (C-6, d), 94.7 (C-8, d), 79.8 (C-G_2, d), 78.8 (C-G_3, d), 78.0 (C-G_5, d), 74.0 (C-R_4, d), 72.3 (C-R_2, d), 72.2 (C-R_3, d), 71.7 (C-G_4, d), 69.9 (C-R_5, d), 62.6 (C-G_6, t), 17.6 (C-R_6, q).

Kaempferol 3-*O*-β-D-glucopyranoside (**4**), yellow powder; negative ESI-MS, *m/z* 447 $[M-H]^-$; ^1H NMR (CD_3OD): δ_H 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-G_1), 3.69 (1H, dd, *J* = 12, 1.8 Hz, H-G_{6a}), 3.52 (1H, dd, *J* = 9, 7.8 Hz, H-G_2), 3.41 (2H, m, H-G_3, G_{6b}), 3.30 (1H, brdd, *J* = 9, 5.4 Hz, H-G_5), 3.20 (1H, t, *J* = 9 Hz, H-G_4). ^{13}C NMR (CD_3OD): δ_C 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-G_1, d), 95.2 (C-8, d), 78.4 (C-G_2, d), 78.0 (C-G_5, d), 75.7 (C-G_3, d), 71.3 (C-G_4, d), 62.5 (C-G_6, t).

Kaempferol- 3, 7-di-*O*-β-D-glucopyranoside (**5**): yellow powder; negative ESI-MS, *m/z* 609 $[M-H]^-$; ^1H NMR (C_5D_5N): δ_H 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-G_1), 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-G′$_5$), 4.40 (1H, m, H-G_{6b}), 4.35 (1H, m, H-G′$_3$), 4.33 (1H, m, H-G′$_2$), 4.26 (1H, m, H-G_2), 4.25 (1H, m, H-G_3), 4.19 (2H, m, H-G_4, G′$_4$), 4.04 (1H, m, H-G_5). ^{13}C NMR (C_5D_5N): δ_C 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-G′$_1$, s), 100.3 (C-6, d), 99.0 (C-G_1, s), 94.7 (C-8, d), 77.9 (C-G_5, G′$_5$, d), 77.2 (C-G_3, d), 77.1 (C-G′$_3$, d), 74.7 (C-G_2, d), 73.4 (C-G′$_2$, d), 70.1 (C-G_4, d), 69.7 (C-G′$_4$, d), 61.2 (C-G_6, t), 61.0 (C-G′$_6$, t).

7-*O*-β-D-glucopyranosyl kaempferol 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**6**): yellow powder; negative ESI-MS, *m/z* 753 $[M-H]^-$; ^1H NMR (CD_3OD): δ_H 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-G_1), 5.24 (1H, brs, H-R_1), 5.05 (1H, d, *J* = 7.2 Hz, H-G′$_1$), 4.07 (1H, m, H-R_5), 4.06 (1H, dd, *J* = 8.4, 7.2 Hz, H-G′$_2$), 4.05 (1H, m, H-R_2), 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-G’$_3$), 3.62 (1H, ddd, *J* = 12, 8.4, 2.4 Hz, H-G′$_5$), 3.60 (1H, dd, *J* = 9.6, 7.2 Hz, H-G_2), 3.55 (1H, t, *J* = 9.6 Hz, H-G_3), 3.44 (1H, t, *J* = 8.4 Hz, H-G′$_4$) 3.38 (1H, m, H-R_4), 3.37 (1H, brd, *J* = 10.2 Hz, H-G_{6b}), 3.34 (1H, t, *J* = 9.6 Hz, H-G_4), 3.29 (1H, m, H-G_5), 1.0 (1H, d, *J* = 6 Hz, H-R_6). ^{13}C NMR (CD_3OD): δ_C 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-R_1, d), 101.5 (C-6, d), 100.6 (C-G_1, d), 100.3 (C-G′$_1$, d), 95.7 (C-8, d), 79.9 (C-G_2, d), 78.8 (C-G_3, d), 78.2 (C-G_5, G′$_5$, d), 77.7 (C-G′$_3$, d), 74.6 (C-G′$_2$, d), 74.0 (C-R_4, d), 72.3 (C-R_2, d), 72.2 (C-R_3, d), 71.8 (C-G_4, d), 71.2 (C-G′$_4$, d), 69.9 (C-R_5, d), 62.6 (C-G_6, t), 62.4 (C-G′$_6$, t), 17.6 (C-R_6, q).

Kaempferol 3-*O*-α-L-rhamnopyranosyl (1→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): δ_H 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-G_1), 5.32 (1H, d, J = 7.8 Hz, H-G'$_1$), 5.23 (1H, brs, H-R_1), 5.11 (1H, dd, J = 9, 7.8 Hz, H-G'$_2$), 4.02 (1H, m, H-R_5), 4.00 (1H, m, H-R_2), 3.95 (1H, dd, J = 11.4, 1.8 Hz, H-G'$_{6a}$), 3.82 (2H, m, H-R_3, 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-G_2), 3.54 (1H, t, J = 9 Hz, H-G_3), 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-R_4), 3.27 (1H, H-4, t, J = 9.0 Hz, H-G_4), 3.23 (1H, ddd, J = 9.0, 4.8, 2.4 Hz, H-G_5), 0.95 (3H, d, J = 6 Hz, H-R_6). ^{13}C NMR (CD_3OD): 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-R_1, d), 100.4 (C-G_1, d), 100.0 (C-6, d), 99.6 (C-R_1, d), 95.8 (C-8, d), 79.8 (C-G_2, d), 78.5 (C-G_3, d), 78.0 (C-G′$_5$, d), 77.8 (C-G_5, d), 75.5 (C-G′$_3$, d), 74.4 (C-G′$_2$, d), 73.4 (C-R_4, d), 71.9 (C-R_2, d), 71.8 (C-R_3, d), 71.5(C-G_4, d), 71.0 (C-G'$_4$, d), 69.5 (C-R_5, d), 62.2 (C-G_6, t), 61.9 (C-G′$_6$, t), 17.5 (C-R_6, 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→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→2)-β-D-glucopyranoside (**6**), 7-*O*-[2-*O*-(*trans*-*p*-coumaroyl)]-β-D-glucopyranosyl kaempferol 3-*O*-α-L-rhamnopyranosyl (1→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 $C_{29}H_{32}O_{16}$ determined by negative-ion ESI-MS (at m/z 635 $[M-H]^-$) as well as ^{13}C NMR data. Its ^{1}H and ^{13}C NMR spectra (Table 1) showed the signals for kaempferol. The ^{1}H 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 ^{13}C 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-

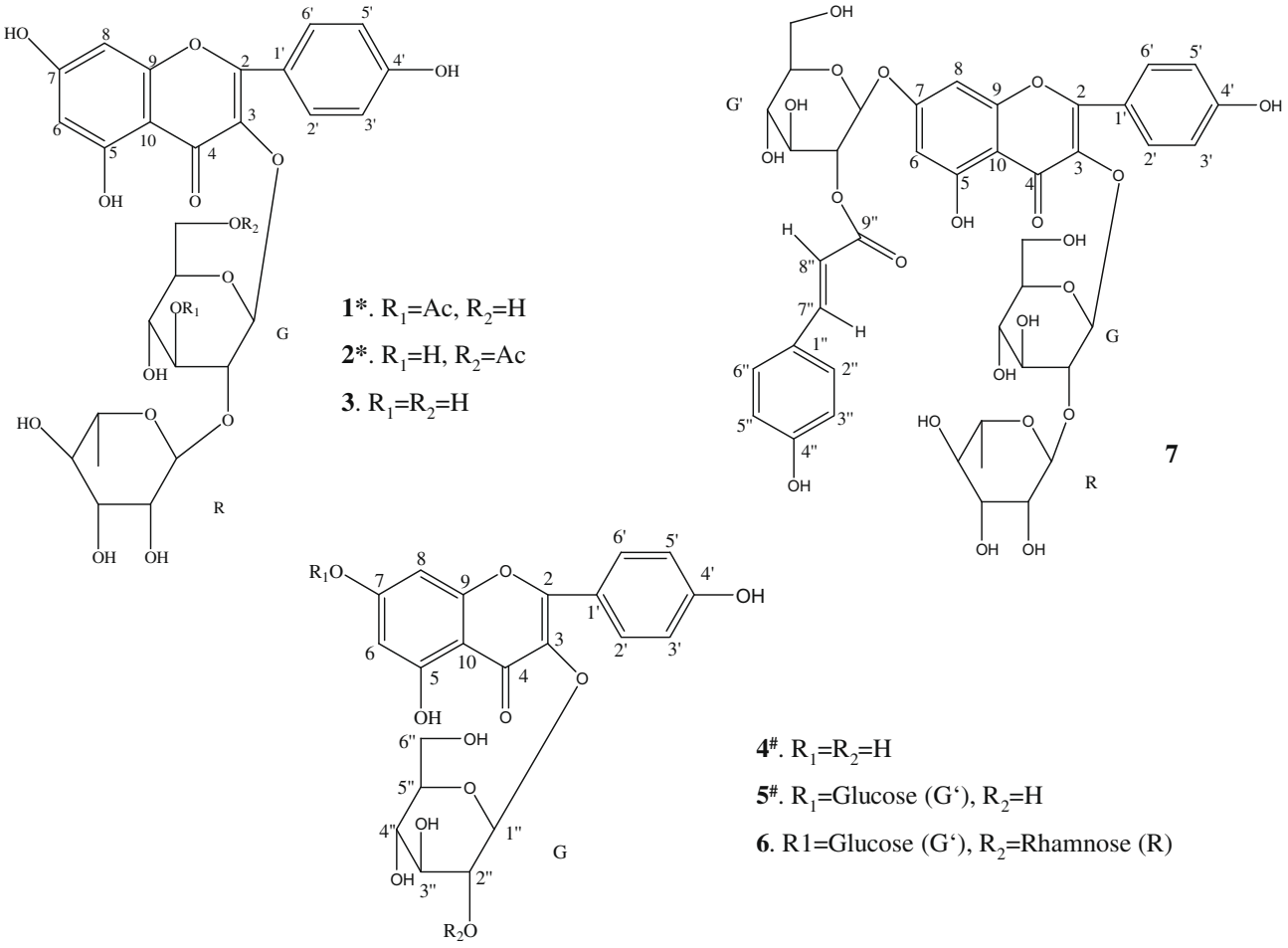

Fig. 1. Structures of compounds **1-7,** kaempferol 3-*O*-α-L-rhamnopyranosyl (1→2)-[3-*O*-acetyl]-β -D-glucopyranoside (**1**), kaempferol 3-*O*- α -L -rhamnopyronosyl (1→2)-[6-*O*-acetyl]-β-D-glucopyranoside (**2**), kaempferol 3-*O*- α -L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**3**), kaempferol 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→2)-β-D-glucopyranoside (**6**), kaempferol 3-*O*- α -L-rhamnopyranosyl (1→2)- β -D-glucopyranoside-7-*O*-[2-*O*-(*trans*-*p*-coumaroyl)]-β-D-glucopyranoside (**7**), (∗ novel compounds and # compounds first isolated from *Allium ursinum*).

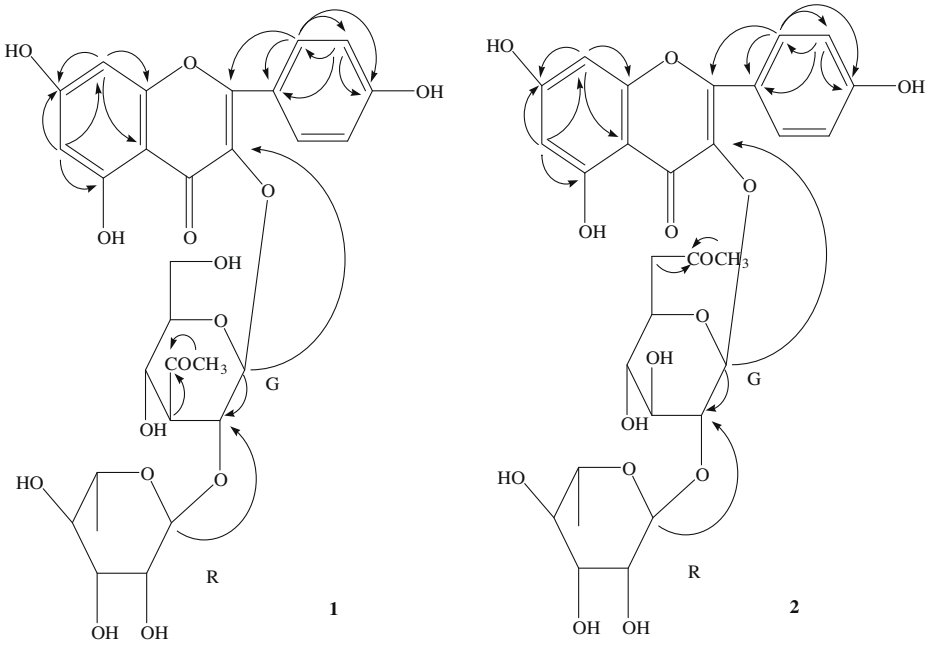

Fig. 2. Significant HMBC (H→C) correlations of compounds **1** and **2**.

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 $^{3}J_{H1,\ H2}$ 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-G_1 (δ 5.82), C-G_2 (δ 79.0) and H-R_1 (δ 4.87) (Fig. 2). Therefore, the glucose unit located at the C-3 position of kamepferol, and the rhamnose unit connected at the C-2 position of the glucose.

In comparison to the ^{1}H 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 monoacetylated derivative of compound **3**. The HMBC spectrum showed the cross peak between δ_C 172.3 and H-G_3 (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 $C_{29}H_{32}O_{16}$, as noted above for compound **1**. The NMR spectra of **2** displayed signal patterns similar to those of **1** (Table 1). The ^{1}H 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-G_1 (δ 5.59), C-G_2 (δ 79.8) and H-R_1 (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-G_6 (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.

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