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FLAVONOIDS FROM THE LEAVES OF *Actinidia kolomikta*

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*One new acetylated flavonoid, kaempferide-7-*O*-(4″-*O*-acetyl)-α-L-rhamnoside, and six known flavonoids were isolated from the EtOAc fraction of the leaves of* Actinidia kolomikta *(Rupr. et Maxim.) Planch. The chemical structures of the isolated compounds were established by application of chemical and spectroscopic analysis. The new acetylated flavonoid was screened for its protective effect on human erythrocytes against AAPH-induced hemolysis, and it can slow the hemolysis induced by AAPH.*

Keywords: *Actinidia kolomikta*, Actinidiaceae, acetylated flavonoid, AAPH-induced hemolysis.

Actinidia kolomikta (Rupr. et Maxim.) Planch, belonging to the liane family of Actinidiaceae, is mainly distributed in China, North Korea, Japan, Russia and so on, especially in Changbai Mountain of China. *Actinidia kolomikta* is usually called "gouzaozi" locally, and its leaves and fruits can be used as medicine [1]. Flavonoids, leucoyandia, lactones, and saponarins have been found in the leaves of *Actinidia kolomikta* [2]. Previous phytochemical investigations on the leaves of *Actinidia kolomikta* resulted in the isolation of several flavonol glycosides [3, 4]. In our ongoing investigation of biologically active compounds from this plant, an acetylated flavonol glycoside **1**, along with six known flavonol glycosides **2–7**, were isolated for the first time from the EtOAc fraction of the EtOH extract of the leaves of *Actinidia kolomikta.* The present paper describes the isolation, structure elucidation, and protect effect of the new acetylated flavonol glycoside on human erythrocytes against AAPH-induced hemolysis.

Compound **1**, was obtained as a yellow powder. The UV spectrum exhibited λ_{max} at 267 and 366 nm characteristic for a flavonol skeleton. The IR spectrum of compound **1** showed the bands for the hydroxyl group at 3413 cm^{-1}, the carbon group at 1723 cm^{-1}, aromatic rings at 1655 and 1608 cm^{-1}, and the glycoside C-O at 1080 cm^{-1}. The high-resolution (HR) ESI-MS gave an ion at *m/z* 511.12163 $[M + Na]^+$, suggesting that the molecular formula is $C_{24}H_{24}O_{11}$ (calcd for $C_{24}H_{24}O_{11}$: 488.13186). The ^1H NMR experiment showed a two-proton doublet for H-2′ and H-6′ at δ_H 8.17 (2H, d, J = 8.4 Hz), coupled to another doublet at δ 7.11 (2H, d, J = 8.4 Hz) for H-3′ and H-5′; H-6 and H-8 appeared at δ 6.46 (1H, d, J = 1.8 Hz) and δ 6.87 (1H, d, J = 1.8 Hz), which was characteristic for the aglycone pattern of kaempferol derivatives. The proton signals at δ 3.87 (3H, s) in the ^1H NMR spectrum and the carbon signal at δ 55.54 suggested the presence of an aromatic methoxyl group, and this methoxyl group was located at C-4′ for the HMBC correlation between the proton at δ 3.86 (OCH_3) and the carbon at δ 160.82 (C-4′) (Fig. 1). The anomeric proton at δ 5.62 (1H, d, J = 1.2 Hz, H-1″), the proton signals at δ 1.017 (3H, d, J = 6 Hz, H-6″) in the ^1H NMR experiment, and the carbons at δ 97.98 (C-1″) and δ 17.64 (C-6″) in the ^{13}C NMR experiment suggested the present of a rhamnosyl unit. Since the coupling constant of the anomeric proton of glucosyl was 1.2 Hz, we believe that the rhamnosyl has an *α*-pyranoside configuration. The ^1H–^1H COSY and HMQC experiments of compound **1** led to unambiguous assignments of signals of the protons and protonated carbons in the NMR spectra. The chemical shift of C-3 (δ_C 136.50), C-2 (δ_C 147.02) and C-7 (δ_C 161.23) suggested that the kaempferide aglycone was glycosylated at C-7, which was confirmed by a long-range correlation from H-1″ to C-7 in the HMBC experiment (Fig. 1). An acetyl singlet at δ 2.04 (3H, s) and δ 170.23 suggested that compound **1** was an acetylated flavonoid. In addition, the carbonyl (δ 170.23) of the acetoxyl group was correlated to H-4″ of the rhamnosyl at δ 4.90 (1H, t, J = 9.6 Hz) (Fig. 1), demonstrating that the acetoxyl group was located at C-4″. Thus, the structure of compound **1** was established as kaempferide-7-*O*-(4″-*O*-acetyl)-*α*-L-rhamnoside.

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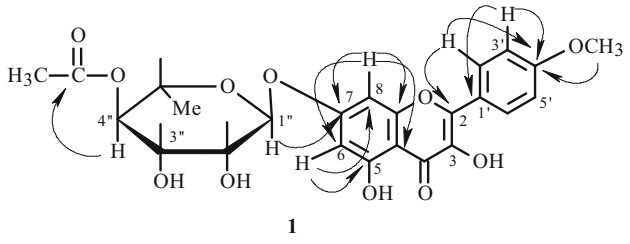
TABLE 1. NMR Data and Correlated Relations in 2D NMR Spectra of Compound **1** (DMSO-d_6, δ, ppm, J/Hz)

C atom	δ_C	δ_H (HMQC)	δ_H (HMBC)	δ_H (^1H–^1H COSY)
2	147.02	–	2′, 6′	–
3	136.50	–	–	–
4	172.23	–	–	–
5	160.28	–	6	–
6	99.04	6.46 (1H, d, J = 1.8)	8	–
7	161.23	–	6, 8, 1″	–
8	94.73	6.87 (1H, d, J = 1.8)	6	–
9	155.93	–	8	–
10	104.96	–	6	–
1′	123.18	–	2′, 3′, 6′, 5′	–
2′	129.64	8.17 (2H, d, J = 8.4)	3′, 6′	3′
3′	114.23	7.11 (2H, d, J = 8.4)	2′, 5′	2′
4′	160.82	–	2′,3′,6′,5′, -OCH_3	–
5′	114.23	7.11 (2H, d, J = 8.4)	3′, 6′	6′
6′	129.64	8.17 (2H, d, J = 8.4)	2′, 5′	5′
OCH_3	55.54	3.86 (3H, s)	–	–
1″	97.98	5.62 (1H, d, J = 1.2)	2″, 3″,5″	2″
2″	69.74	3.91 (1H, s)	1″, 3″, 4″	1″, 3″
3″	67.78	3.63 (1H, m)	1″, 2″, 4″, 5″	2″, 4″
4″	73.39	4.90 (1H, t, J =9.6)	2″, 3″, 5″	3″, 5″
5″	67.65	3.87 (1H, m)	1″, 3″, 4″, 6″	4″
6″	17.64	1.02 (3H, d, J = 6.0)	4″, 5″	5″
>C=O	170.23	–	CH_3-, 4″	–
CH_3	21.06	2.04 (3H, s)		



1

Fig. 1

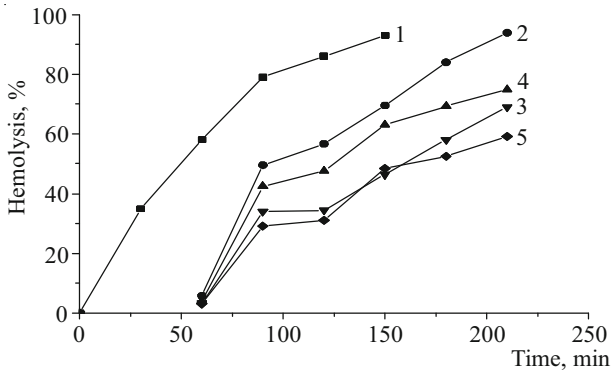


Fig. 2

Fig. 1. The main HMBC correlation of compound **1**.

Fig. 2. AAPH–induced hemolysis of 2.0% human erythrocyte in PBS (pH = 7.4) at 37°C. The final concentrations of the new acetylated flavonoid are: 0 μM (1), 20 μM (2), 40 μM (3), 60 μM (4), 80 μM (5), and the final concentration of AAPH is 30 mM.

Six known compounds **2–7** were identified as kaempferol-7-*O*-α-L-rhamnoside (**2**) [5], kaempferol-3-*O*-β-D-galactoside (**3**) [6], quercetin-3-*O*-β-D-glucoside (**4**) [6], isorhamnetin-3-*O*-β-D-glucoside (**5**) [7], kaempferol-7-*O*-α-L-rhamnosyl-3-*O*-rutinoside (**6**) [8], and kaempferol-3-*O*-[α-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside] (**7**) [9]. These compounds were all isolated for the first time from the leaves of *Actinidia kolomikta.*

We also investigated the antioxidant effect of the new acetylated flavonol glycoside on the experimental system of free-radical-initiated peroxidation: the hemolysis of human erythrocyte induced by a water-soluble initiator, 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH). From Fig. 2, we can see that the hemolysis was slowed at all concentrations.

EXPERIMENTAL

General Methods. Optical rotations were measured on a JASCO P1020 digital polarimeter. Melting points were determined with a Koffler apparatus and were uncorrected. UV spectra were obtained in MeOH using a Unico UV-2102 PCS UV-Vis spectrophotometry, IR Spectra was recorded in KBr disks on a Nicolet 330 Fourier transform infrared spectrometer. NMR spectrum were obtained with a Bruker AV600 (600 MHz) spectrometer using DMSO-d_6 as solvent; chemical shifts are given in ppm with tetramethylsilane (TMS) as an internal standard. High-resolution mass spectrometry was performed on an IonSpec Ultima 7.0 T FTICR mass spectrometer (IonSpec, Lake Forest, CA, USA) with an ESI source in the negative ion mode. HPLC was performed with an Agilent 1200 system using an Eclipse XDB-C_{18} column (5 μm, 4.6 × 250 mm). Preparative HPLC separations were carried out on a Shimadzu liquid chromatograph LC-8A using an SPD-10Avp UV-detector and Phenomenex column (21.20 × 250 mm, 15 μm, C_{18}). Column chromatograpy was carried out on silica gel (200–300 mesh, Qingdaao Haiyang Chemical Co., Qingdao, China), Polyamide (60–80 mesh, TaizhouLuqiao Sijia chemical Co., Taizhou, China), and Sephadex LH-20 (Merck, Germany). TLC was carried out on silica gel (Merck silica gel $60F_{254}$), with the spots visualized under UV light at 254 and/or 365 nm, or by heating silica gel plates after spraying with chromogenic agent (10% H_2SO_4–CH_3CH_2OH).

Plant Material. The leaves of *Actinidia kolomikta* were collected in Jingyu county in Changbai Mountain of China in June 2006 and identified by Prof. Minglu Deng at ChangChun University of Chinese Medicine in which a voucher specimen (No. JLU-AK-2001-06) was deposited.

Extraction and Isolation. The dried, powdered leaves (2.0 kg) were refluxed twice with EtOH (1.2 L,1.0 L) for 2 h, 1.5 h at 80°C. The extract was concentrated under reduced pressure before being suspended in water, then partitioned consecutively with $CHCl_3$, EtOAc, and *n*-butanol. The EtOAc fraction (10 g) was subjected to Si-gel column chromatograpy (CC) and eluted by EtOAc–CH_3OH gradient (20:1 to 10:1) to give three fractions (Fr. A, Fr. B, Fr. C). Fraction A (200 mg) was purified by CC on Si-gel eluting with $CHCl_3$–CH_3OH (10:1) to yield unpurified compound **1**, then recrystallized with CH_3OH to give purified compound **1** (50 mg). Fraction B (5 g) was subjected to a series of purification steps using Si-gel, Sephadex LH-20, polyamide, and an RP-18 column chromatograpy to give compound **2** (100 mg), compound **3** (78 mg), compound **4** (100 mg), and compound **5** (100 mg). Fraction C (150 mg) was subjected to preparative HPLC using a solvent system of MeOH–H_2O (60:40) to give compound **6** (50 mg) and compound **7** (50 mg).

Compound 1. Yellow powder; mp 250–251°C (CH_3OH); $[\alpha]_D^{17}$ –148.634° (*c* 0.183; MeOH); UV (MeOH, λ_{max}, nm): 267, 366 (log ε 3.25, 2.87); IR (KBr, ν, cm^{-1}): 3413, 2930, 1723, 1655, 1608, 1511, 1456, 1351, 1260, 1182, 1080, 836; HR-ESI-MS *m/z:* 511.12163 $[M + Na]^+$; calcd for $C_{24}H_{24}O_{11}$, 488.13186. ^1H NMR and ^{13}C NMR data, see Table 1.

Acid Hydrolysis of Compound 1. A solution of compound **1** (10 mg) in 5% H_2SO_4–MeOH (1:1) was heated at 88°C for 2 h. The reaction mixture was diluted with H_2O and extracted with EtOAc. The EtOAc layer was purified by passing through a Si gel CC by elution with $CHCl_3$–CH_3OH (20:1) to give kaempferide (4.2 mg), which was confirmed by NMR data and co-HPLC with an authentic sample [8]. The water layer was neutralized with saturated aqueous $NaHCO_3$ solution, and the precipitate was filtered off. The filtrate was concentrated to dryness to give 3 mg L-rhamnose, which was identified by NMR data comparison with the literature [10] data and co-TLC with an authentic sample.

Evaluation of Protective Effect on Human Erythrocytes. The hemolysis induced by AAPH was performed according to the literature [11, 12]. Before the experiment, the erythrocytes were washed three times with phosphate-buffered saline (PBS: 138 mM NaCl, 6.1 mM Na_2HPO_4, 1.4 mM KH_2PO_4, 5 mM KCl, pH 7.4). The new acetylated flavonoid was dissolved in DMSO-d_6, then the solution was added to a 2.0% (v/v) suspension of human erythrocytes in PBS (pH 7.4) and incubated at 37°C for 5 min, into which an AAPH (PBS) solution was injected to initiate hemolysis. In every experiment, the concentration of AAPH was kept at 30 $mM \cdot L^{-1}$. The percentage of hemolysis at different incubation intervals was determined by measuring the absorbance of the supernatant of the erythrocytes at 540 nm of the visible spectrum and compared with that of complete hemolysis; the trend of hemolysis is listed in Fig. 2.

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