Acylated Flavonol Glycosides with Anti-complement Activity from *Persicaria lapathifolia*

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During a search for biologically active compounds from traditional medicines, a crude extract of *Persicaria lapathifolia* was found to have anti-complement activity. Bioassay-guided chromatographic separation of the active constituents led to the isolation of a new acylated kaempferol glycoside (1) and three known acylated quercetin glycosides (2—4). The structures of compounds 1—4 were characterized as kaempferol 3-*O*-β-D-(6″-*p*-hydroxybenzoyl)-galactopyranoside, quercetin 3-*O*-β-D-(6″-feruloyl)-galactopyranoside, quercetin 3-*O*-β-D-(2″-galloyl)-rhamnopyranoside and quercetin 3-*O*-β-D-(2″-galloyl)-glucopyranoside, respectively. Compounds 1—4 showed strong anti-complement activity (IC_{50} values of 4.3, 9.7, 3.9 and 7.6×10^{-5} M, respectively) on the classical pathway of the complement. On the other hand, six isolated flavonol glycosides (5—10) did not show any activity on this system.

Key words *Persicaria lapathifolia*; anti-complement activity; kaempferol 3-*O*-β-D-(6″-*p*-hydroxybenzoyl)-galctopyranoside; quercetin 3-*O*-β-D-(6″-feruloyl)-galactopyranoside; quercetin 3-*O*-β-D-(2″-galloyl)-rhamnopyranoside; quercetin 3-*O*-β-D-(2″-galloyl)-glucopyranoside

The complement system is a humoral effector of inflammation and is activated by a cascade mechanism through an antigen–antibody mediated process (classical pathway, CP) and/or antibody independent process (alternative pathway, AP).[1] Activation of the system normally plays a significant role in promoting humoral immune responses. However, excessive complement activation provokes pathological reactions including various degenerative diseases and hyperacute rejection in transplantation.[2] Therefore, modulation of complement activity should be useful in the therapy of inflammatory diseases.

During the screening of plant extracts, the complement-inhibiting properties of the MeOH extract of *Persicaria lapathipolia* Gray (Polygonaceae) were investigated. This plant and another *Persicaria* species, *P. hydropiper*, have been used as an analgesic as well as for the treatment of bleeding.[3] Recently, we reported the superoxide production inhibiting effect of galloylated and feruloylated flavonol glycosides.[4] In this paper we report the isolation of a new acylated kaempferol glycoside and anti-complement activity of the acylated flavonol glycosides from *P. lapathifolia*.

The MeOH extract of *P. lapathifolia* was suspended in water and then consecutively partitioned with $CHCl_3$, EtOAc and BuOH. The EtOAc fraction showed strong anti-complement activity (IC_{50} value of 23.5 μg/ml) and activity guided separation yielded four active acylated flavonol glycosides (**1**—**4**) and six inactive flavonol glycosides (**5**—**10**). Compound **3** showed the most potent inhibitory effect ($IC_{50}=3.9\times10^{-5}$ M) on the CP of the complement system. Other acylated flavonol glycosides, **1**, **2** and **4**, also showed higher activity than that of rosmarinic acid, well known anti-complement material (Table 1). On the other hand, kaempferol glycosides **5**—**7** and quercetin glycosides **8**—**10** as well as components of the active acylated compounds (*e.g.* quercetin, kaempferol, ferulic acid, gallic acid, sugars) did not show the anti-complement activity up to 2×10^{-4} M. Jung *et al.* reported that kaempfrol 3-*O*-β-D-(6″-*p*-coumaroyl)-glucopyranoside had strong anti-complement activity ($IC_{50}=5.4\times10^{-5}$ M), but its hydrolysates, kaempfrol, astragalin and *p*-coumaric acid, showed very weak activity.[7] These observations indicate that the configuration of flavonol–sugar–aromatic side chain is essential for potent anti-complement activity, where the types of flavonols, sugars and aromatic side chains are less important.

The molecular formula of **1** was established as $C_{28}H_{24}O_{13}$ by high resolution-FAB mass spectrometry. In the IR spectrum, signals for hydroxyl (3420 cm^{-1}), ester carbonyl (1660 cm^{-1}), conjugated carbonyl (1605 cm^{-1}) groups were apparent. The ^{1}H-NMR spectrum suggested that **1** has a kaempferol moiety. Signals at δ 6.20 (1H, d, J=1.7 Hz) and 6.38 (1H, d, J=1.7 Hz) are characteristic of a 5,7-disubstituted A ring, and signals at δ 6.85 (2H, d, J=8.8 Hz) and 8.02 (2H, d, J=8.8 Hz) of a 4′-monosubstituted B ring of a kaempferol moiety. Doublets at δ 6.67 (2H, d, J=8.6 Hz) and 7.54 (2H, d, J=8.6 Hz) were originated from the *p*-hydroxybenzoyl moiety. In the heteronuclear multiple bond connectivity (HMBC) spectrum, doublets at δ 8.02 and 6.85 showed cross peaks with signals of C-2 (δ 156.1) and C-1′ (δ 120.8), respectively. The other doublets at δ 7.54 and 6.67 showed cross peaks with a signal of ester carbonyl carbon (C-7‴ at δ 165.2) and of C-1‴ (δ 120.1), respectively (Fig. 1). These re-

Table 1. IC_{50} Values of Extracts from *P. lapathifolia*, Flavonoids and Rosmarinic Acid on the CP of the Complement System

Compound	IC_{50} value
MeOH extract	28.0 μg/ml
EtOAc fraction	23.5 μg/ml
1	4.3×10^{-5} M
2	9.7×10^{-5} M
3	3.9×10^{-5} M
4	7.6×10^{-5} M
Rosmarinic acid[a)]	1.8×10^{-4} M

a) This compound was used as a positive control.

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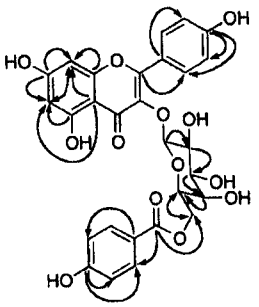
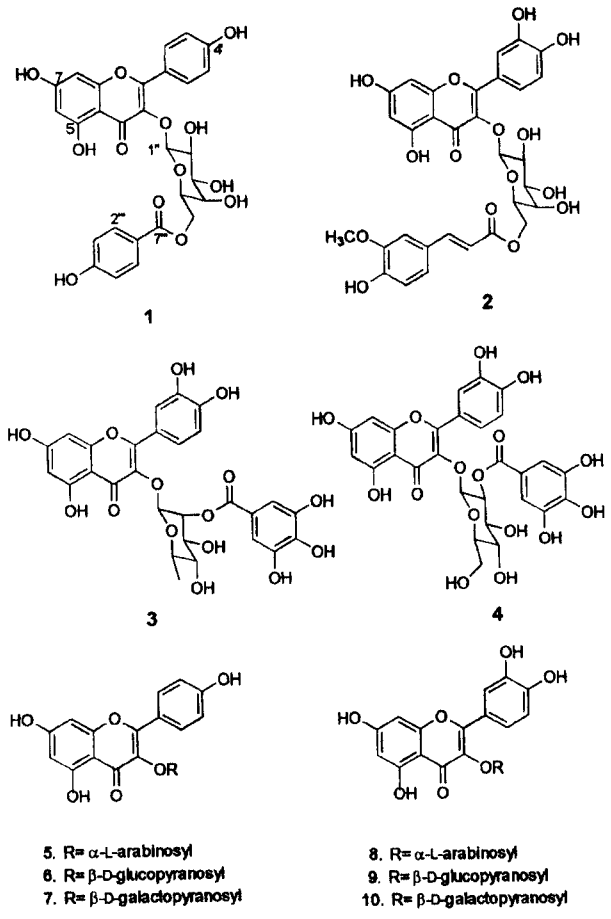


Fig. 1. HMBC of Kaempferol 3-*O*-*β*-D-(6″-*p*-Hydroxybenzoyl)-galactopyranoside (**1**) from *Persicaria lapathifolia*



5. R= α-L-arabinosyl
6. R= β-D-glucopyranosyl
7. R= β-D-galactopyranosyl
8. R= α-L-arabinosyl
9. R= β-D-glucopyranosyl
10. R= β-D-galactopyranosyl

sults confirmed the presence of a B ring of kaempferol and a *p*-hydroxybenzoyl moiety. The ^{1}H-NMR spectrum showed a characteristic signal assignable to an anomeric proton at δ 5.47 (1H, d, J=7.6 Hz). In the ^{13}C-NMR spectrum, the downfield shift of a methylene carbon (C-6″ at δ 63.4) along with the upfield shift of a neighboring carbon (C-5″ at δ 73.0) of a galactose indicated that the *p*-hydroxybenzoyl group is attached at C-6″ of the galactose.[6] In the HMBC spectrum, cross peaks between signals of an ester carbonyl carbon and H-6″ protons (δ 4.15) confirmed the ester linkage between C-6″ of galactose and C-7‴ of a *p*-hydroxybenzoyl moiety. On the basis of the above observations, compound **1** was assigned as kaempferol 3-*O*-*β*-D-(6″-*p*-hydroxybenzoyl)-galactopyranoside.

Table 2. ^{1}H- (300 MHz, DMSO-d_6) and ^{13}C-NMR (75 MHz) Data of Kaempferol 3-*O*-*β*-D-(6″-*p*-hydroxybenzoyl)-galactopyranoside (**1**)

No	H	C
2		156.1
3		133.0
4		177.4
5		161.1
6	6.20 (1H, d, 1.7 Hz)	98.9
7		164.4
8	6.38 (1H, d, 1.7 Hz)	93.6
9		156.3
10		103.6
1′		120.8
2′, 6′	8.02 (2H, d, 8.8 Hz)	130.8
3′, 5′	6.85 (2H, d, 8.8 Hz)	115.0
4′		159.9
1″	5.47 (1H, d, 7.6 Hz)	101.3
2″	3.56 (1H, m)	71.0
3″	3.44 (1H, m)	72.9
4″	3.70 (1H, m)	68.3
5″	3.74 (1H, m)	73.0
6″	4.15 (2H, m)	63.4
1‴		120.1
2‴, 6‴	7.54 (2H, d, 8.6 Hz)	131.0
3‴, 5‴	6.67 (2H, d, 8.6 Hz)	115.1
4‴		161.8
7‴		165.2

The structure of compound **2** was reported in the previous paper as quercetin 3-*O*-*β*-D-(6″-feruloyl)-galactopyranoside.[4] NMR data of compounds **3**—**10** were identical with the reported data and thus assigned as quercetin 3-*O*-*β*-D-(2″-galloyl)-rhamnopyranoside (**3**), quercetin 3-*O*-*β*-D-(2″-galloyl)-glucopyranoside (**4**), kaempferol 3-*O*-*α*-L-arabinopyranoside (**5**), kaempferol 3-*O*-*β*-D-glucopyranoside (**6**), kaempferol 3-*O*-*β*-D-galactopyranoside (**7**), quercetin 3-*O*-*β*-L-arabinopyranoside (**8**), quercetin 3-*O*-*β*-D-glucopyranoside (**9**) and quercetin 3-*O*-*β*-D-galactopyranoside (**10**), respectively.[7]

Experimental

General procedure Silica gel (230—400 mesh) was purchased from Merck Co. (Germany) and reversed phase (RP-18) silica gel (70—230 mesh, YMC GEL ODS-A) was purchased from YMC Co. (Japan). Sephadex LH-20 was purchased from Sigma (U.S.A.).The ^{1}H-NMR (300 or 600 MHz) and ^{13}C-NMR (75 or 150 MHz) spectra were obtained using Varian Unity 300 or Bruker DRX-600 spectrometer. The FAB-MS spectra were measured with JEOL JMS-HX 110A tandem mass spectrometer. IR spectrum was recorded on KBr disc.

Determination of Anti-complement Activity through the Classical Pathway Anti-complement activity was determined by the modified method of Mayer as described previously.[8] For the CP assay, a diluted solution of normal human serum (80 μl) was mixed with gelatin veronal buffer (80 μl) with or without the sample. The mixture was preincubated at 37 °C for 30 min, then sensitized sheep red blood cells (40 μl) were added. After incubation under the same conditions, the mixture was centrifuged and the optical density of the supernatant (100 μl) was measured at 405 nm. Anti-complement activity was determined as a mean of triplicates.

Isolation Procedure The dried and chopped plant (1.5 kg) was extracted with MeOH (10 l×3), then the MeOH extracts were concentrated to give a residue (120 g). The residue was suspended with water and successively partitioned with $CHCl_3$, EtOAc and BuOH. The EtOAc extract (20 g) was loaded on a reverse phase C-18 column (4.5×40 cm) and eluted with MeOH–H_2O (1 : 1), (2 : 1) then 100% MeOH. Fraction 3 was subjected to a Sephadex LH-20 column (3×50 cm) eluted with MeOH–H_2O (3 : 1) and yielded quercetin 3-*O*-*β*-D-(2″-galloyl)-glucopyranoside (**4**) (450 mg). Fraction 5 was chromatographed on a silica gel column (3×50 cm) with $CHCl_3$–MeOH (9 : 1) then the polarity of the solvent was increased to $CHCl_3$–MeOH (1 : 2). Kaempferol 3-*O*-*β*-L-arabinopyranoside (**5**) (35 mg),

kaempferol 3-*O*-β-D-galactopyranoside (**7**) (142 mg) and quercetin 3-*O*-β-D-(2″-galloyl)-rhamnopyranoside (**3**) (150 mg) were isolated from the subfraction 5-1, 5-5 and 5-7, respectively. Subfraction 5-4 was subjected to a Sephadex LH-20 column (2×50 cm) eluted with MeOH–H_2O (3 : 1) and yielded kaempferol 3-*O*-β-D-glucopyranoside (**6**) (130 mg) and quercetin 3-*O*-β-D-(2″-feruloyl)-galactopyranoside (**2**) (30 mg). Quercetin 3-*O*-β-D-glucopyranoside **9** (85 mg) was isolated as a crystal from subfraction 5-8, and quercetin 3-*O*-β-D-galactopyranoside (**10**) (35 mg) was also isolated from the same subfraction after Sephadex LH-20 column chromatography. Quercetin 3-*O*-β-L-arabinopyranoside **8** (48 mg) was purified from subfraction 5-4-7 by Sephadex LH-20 column (2×50 cm) using MeOH as a eluting solvent. Kaempferol 3-*O*-β-D-(6″-*p*-hydroxybenzoyl)-galactopyranoside (**1**) (10 mg) was isolated from subfraction 5-4-5 by repeated Sephadex LH-20 column (2×140 cm) chromatography using MeOH as an eluting solvent.

Kaempferol 3-*O*-β-D-(6″-*p*-hydroxybenzoyl)-galactopyranoside (1) Yellow amorphous powder, UV λ_{max} (MeOH) nm (log ε) 268 (4.50), IR (KBr) cm^{-1}: 3420, 1660, 1605, 1514, 1441, 1175, 1083, FAB-MS *m/z*: 569 $[M+H]^+$, 591 $[M+Na]^+$, HR-FAB-MS *m/z*: 569.1273 ($[M+H]^+$, $C_{28}H_{25}H_{13}$, requires : 569.1295), ^{1}H-NMR and ^{13}C-NMR : Table 2.

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