Anti-complement Activity of Tiliroside from the Flower Buds of *Magnolia fargesii*

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As part of the search for anticomplementary active components from natural products, the anticomplementary properties of methanolic extracts from the flower buds of *Magnolia fargesii* have been investigated. Bioassay-guided chromatographic separation of the active constituents led to the isolation of compound 1, whose structure was identified by spectroscopic methods to be kaempferol 3-*O*-β-D-(6″-*O*-coumaroyl)glucopyranoside (tiliroside). Tiliroside showed very potent anti-complement activity ($IC_{50}=5.4\times10^{-5}$ M) on the classical pathway of the complement system, even higher than rosmarinic acid, which is a well-known inhibitor against the complement system. On the other hand, the hydrolysates of tiliroside, kaempferol, astragalin and *p*-coumaric acid showed very weak activity on this system.

Key words *Magnolia fargesii*; tiliroside; anticomplementary activity

The complement system comprises a group of more than 30 serum and cell surface proteins, and complement activation by a cascade mechanism through classical pathway (CP) and/or alternative pathway (AP) plays a significant role in promoting humoral immune responses. In complement activation, the complement fragments induce the release of mediators from mast cells, causing reactions that resemble immediate hypersensitivity; in extreme cases, this reaction can mimic anaphylaxis. Therefore, the modulation of complement activity can play an important part in inflammation.[1]

As part of a study to detect natural products with anticomplementary activity, the complement-inhibiting properties of the flower buds of *Magnolia fargesii* CHENG (Magnoliaceae) have been investigated. The dried flower buds of *M. fargesii* have been used to treat nasal empyema with headache, sinusitis, and allergic rhinitis.[2] Recently, we reported the isolation and platelet-activating factor (PAF) antagonistic effect of two new lignans, namely magnone A and B, and the isolation of sesquiterpenes.[3,4]

In this paper we report the isolation and anticomplementary activity of tiliroside (**1**), which was isolated for the first time from *M. fargesii*.

MATERIALS AND METHODS

General Experimental Procedures Melting points were measured by an Electrothermal 9100 and are uncorrected. The IR and UV spectra were recorded using a Magna 550 in KBr pellet and a Shimadzu UV-260 spectrophotometer, respectively. The FAB-MS spectra were obtained on a JEOL JMS-HX 110A tandem mass spectrometer. The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a JEOL Lambda 400 spectrometer and chemical shifts were referenced to tetramethylsilane (TMS) as the internal standard. Column chromatography was carried out on Kieselgel 60 (Merck No. 9385 and 7729) and Sephadex LH-20 (Pharmacia Fine Chemicals).

Plant Material The dried flower buds of *M. fargesii* were purchased from Il-Shin Pharm. Co. (Taejon, Korea). The voucher specimen is deposited in our laboratory (NDC-052).

Source of Test Substances Rosmarinic acid, used as an anticomplementary reference material, and astragalin were isolated from *Agastache rugosa* and *Sophora japonica*, respectively, and *o*-, *m*-, and *p*-coumaric acids were purchased from Sigma Co. (St. Louis, U.S.A.).[5]

Extraction and Isolation The dried and pulverized flower buds (18 kg) were extracted with MeOH, then the MeOH extracts were concentrated to give a residue (2.4 kg). The residue was partitioned between EtOAc and water. A part (120 g) of the EtOAc extract (2 kg) was loaded on a silica gel column and eluted with a stepwise solvent gradient of MeOH in $CHCl_3$ to afford fifteen subfractions. The subfraction 12 (2 g) was rechromatographed on a Sephadex LH-20 column using MeOH as an eluent to give six fractions. Fraction 5 was further purified by repeated silica gel column chromatography ($CHCl_3$–MeOH, 99 : 1) to give compound **1** (211 mg).

Tiliroside (1) Yellow amorphous powder from MeOH. mp 267—269 °C. UV λ_{max} (MeOH) nm (log ε) 267 (4.42), 315 (4.52): (+NaOMe) 277 (4.42), 365 (4.60): (+$AlCl_3$) 275 (4.61), 306 (4.56), 399 (4.21): (+$AlCl_3$+HCl) 276 (4.64), 305 (4.58), 394 (4.14): (+NaOAc) 275 (4.61), 312 (4.55), 373 (4.40): (+NaOAc+H_3BO_3) 268 (4.58), 314 (4.60), 360 (sh, 4.40). IR (KBr) cm^{-1}: 3393, 1662, 1605, 1516, 1442, 1361, 1173, 1084, 836. FAB-MS *m/z*: 617 $[M+Na]^+$, 595 $[M+H]^+$. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 3.98 (1H, dd, J=12.0, 6.3 Hz, H-6″a), 4.22 (1H, br d. J= 12.0 Hz, H-6″b), 5.40 (1H, d, J=7.3 Hz, H-1″), 6.06 (1H, d, J=15.8 Hz, H-8‴), 6.10 (1H, d, J=2.0 Hz, H-6), 6.34 (1H, d, J=2.0 Hz, H-8), 6.74 (2H, d, J=8.6 Hz, H-3‴, 5‴), 6.81 (2H, d, J=8.9 Hz, H-3′, 5′), 7.28 (1H, d, J=15.8 Hz, H-7‴), 7.31 (2H, d, J= 8.6 Hz, H-2‴, 6‴), 7.93 (2H, d, J=8.9 Hz, H-2′, 6′), 12.56 (1H, s, 5-OH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ:156.8 (C-2), 133.5 (C-3), 177.8 (C-4), 161.5 (C-5), 99.2 (C-6), 164.6 (C-7), 94.1 (C-8), 156.9 (C-9), 104.3 (C-10), 121.2 (C-1′), 131.2 (C-2′, 6′), 115.5 (C-3′, 5′), 160.2 (C-4′), 101.4 (C-1″), 74.6 (C-2″), 76.6 (C-3″), 70.4 (C-4″), 74.5 (C-5″), 63.4 (C-6″), 125.3 (C-1‴), 130.5 (C-2‴, 6‴), 116.2 (C-3‴, 5‴), 160.4 (C-4‴), 145.0 (C-7‴), 114.0 (C-8‴), 166.6 (C-9‴).

Acid Hydrolysis of 1 A solution of **1** (30 mg) in 5% methanolic H_2SO_4 was refluxed to afford yellow needles,

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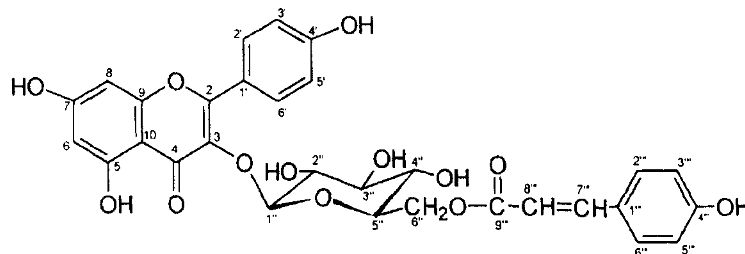

tiliroside (1)

Table 1. IC_{50} Values of Extracts from *M. fargesii*, Flavonoids, Coumaric Acids and Rosmarinic Acid on the Classical Pathway of the Complement System

Compound	IC_{50} value
MeOH extract	36.0 μg/ml
EtOAc extract	56.1 μg/ml
Tiliroside	5.4×10^{-5} M
Kaempferol[a]	$>1\times10^{-3}$ M
Astragalin[b]	$>1\times10^{-3}$ M
o-Coumaric acid	6.0×10^{-4} M
m-Coumaric acid	6.5×10^{-4} M
p-Coumaric acid	5.9×10^{-4} M
Rosmarinic acid[c]	1.8×10^{-4} M

a) 1.9% inhibition at 1 mM. *b*) 27% inhibition at 1 mM. *c*) This compound was used as a positive control.

kaempferol, on cooling. The residue was partitioned between EtOAc and water, and D-glucose from the aqueous phase and *p*-coumaric acid from the EtOAc phase were confirmed by HPLC and co-TLC.

Determination of Anticomplementary Activity through the Classical Pathway Anticomplementary activity was determined by the modified method of Mayer as described previously.[6,7] For the classical pathway assay, a diluted solution of normal human serum (80 μl) was mixed with gelatin veronal buffer (80 μl) with or without 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. Anticomplementary activity was determined as a mean of triplicates.

RESULTS AND DISCUSSION

After repeated column chromatography of the EtOAc-soluble portion of a methanolic extract, **1** was obtained as a yellow amorphous powder. The molecular weight of **1** was determined to be 594 by FAB-MS, showing *m/z* 617 $[M+Na]^+$ and 595 $[M+H]^+$. The UV spectrum in MeOH solution exhibited absorption bands at 267 and 315 nm, and bathochromic shifts on the addition of shift reagents indicated the presence of free hydroxyl groups at C-5, C-7 and C-4′ of the 3-hydroxyl substituted flavonol skeleton. Acid hydrolysis of **1** gave kaempferol, glucose and *p*-coumaric acid, which were identified by direct comparison with authentic standards. The ^{1}H-NMR spectrum of **1** showed signals due to a kaempferol moiety, β-glucose and *p*-coumaroyl ester. Furthermore, the ^{13}C-NMR chemical shift values of **1** for each corresponding carbon were substantially identical with tiliroside. Consequently, **1** was determined to be kaempferol 3-*O*-β-D-(6″-*O*-coumaroyl)glucopyranoside (tiliroside).[8]

An initial MeOH extract of the flower buds of *M. fargesii*, as well as the EtOAc extract from this MeOH extract, inhibited the CP of the complement system in a dose-dependent manner. Activity-guided fractionation of the EtOAc extract was performed based on the effect on CP, from which tiliroside was isolated, and its anticomplementary activity was assessed *in vitro*. As shown in Table 1, tiliroside, composed of astragalin and *p*-coumaric acid, showed a dose-dependent inhibitory effect ($IC_{50}=5.4\times10^{-5}$ M) on the CP of the complement system, which was even higher than that of rosmarinic acid ($IC_{50}=1.8\times10^{-4}$ M) used as a reference material.[9] On the other hand, kaempferol, astragalin and *p*-coumaric acid, which were obtained by the hydrolysis of tiliroside, showed very weak activity. *o*- and *m*-coumaric acid were found to have a weaker effect than rosmarinic acid on CP. In addition, the various mixtures which combined kaempferol or astragalin with each coumaric acid (final concentration of each compound, 500 μM) were inactive in this system (data not shown). Although the structure–activity relationship was not investigated in this study, the configuration of **1** was assumed to contributed to the potency. The present result clearly demonstrates the marked *in vitro* inhibitory activity of tiliroside on the complement system, and the comparison with rosmarinic acid confirms that tiliroside is a potent complement inhibitor.

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