# Antiviral Flavonoid-Type *C*-Glycosides from the Flowers of *Trollius chinensis*

by **Shao-Qing Cai***[a]), **Rufeng Wang**[a]), **Xiuwei Yang***[a]), **Mingying Shang**[a]), **Chaomei Ma**[a]), and **Yukihiro Shoyama**[b])

[a]) The State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100083, P. R. China (phone/fax: +86-10-8280-1693; e-mail: sqcai@bjmu.edu.cn, xwyang@bjmu.edu.cn)

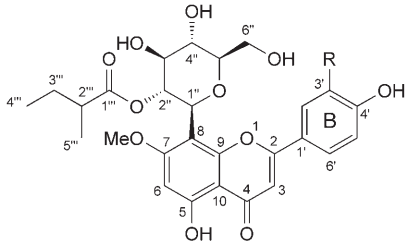
[b]) Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812, Japan

Two new flavonoid-type *C*-glycosides, trollisin I (=(1*S*)-1,5-anhydro-1-[2-(3,4-dihydroxyphenyl)-5-hydroxy-7-methoxy-4-oxo-4*H*-[1]benzopyran-8-yl]-2-*O*-(2-methylbutanoyl)-D-glucitol; **1**) and its 2-*O*-benzoyl congener trollisin II (**2**), were isolated from *Trollius chinensis* BUNGE, together with the two known compounds 2″-*O*-(2‴-methylbutanoyl)isoswertisin (**3**) and vitexin galactoside (**4**). All compounds were identified by HR-ESI-MS and in-depth NMR-spectroscopic analyses. In antiviral assays, compound **3** was found to be moderately active towards influenza virus A.

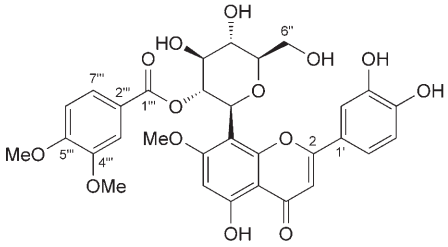
**Introduction.** – The flower of *Trollius chinensis* BUNGE has been used since ancient times for the therapy of respiratory infections, tonsillitis, pharyngitis, and bronchitis in Chinese folk medicine [1]. The crude extract of the flower and some compounds thereof, *e.g.*, vitexin and orientin, were reported to have antibacterial and antiviral activities [2–4]. The major constituents isolated from this flower are phenolic compounds and flavonoids [3–7]. In a previous paper [8], we reported the structure of trolloside, an *O*-glycoside of a phenolic acid. In continuation of this work, we now report the isolation, characterization, and properties of two new flavonoid-type *C*-glycosides: trollisin I (**1**) and trollisin II (**2**). Also isolated were two known compounds: 2″-*O*-(2‴-methylbutanoyl)isoswertisin (**3**) [6] and vitexin galactoside (**4**) [7].

**Results and Discussion.** – 1. *Structure Elucidation*. Compound **1** was obtained as a yellow powder, with a melting point of 152–154°. Its molecular formula was established as $C_{27}H_{30}O_{12}$, requiring 13 degrees of unsaturation, as deduced by negative HR-ESI-MS experiments. Compound **1** tested positive to the *Molish* and *Shinoda* (Mg/HCl) tests, which suggested a flavonoid glycoside. The UV spectrum of **1** showed absorption bands at 269 and 346 nm, in accord with a flavonoid skeleton. The IR spectrum displayed absorption bands for OH (3301), C=O (1740), and aromatic moieties (1606, 1549, 1463 $cm^{-1}$), together with a C–O stretching band (1078 $cm^{-1}$), which further corroborated that **1** was a glycoside of a flavonoid.

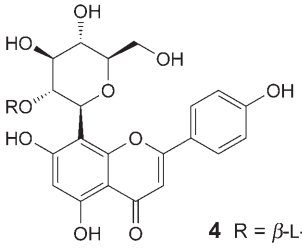
Combined analysis of the $^1$H- and $^{13}$C-NMR (DEPT) spectra revealed that **1** comprised three Me, two $CH_2$, and eleven CH groups, as well as eleven quaternary C-atoms (*Table 1*). The $^1$H-NMR spectrum showed an *ABX* system for ring *B* [$\delta$(H) 6.89



**1** R = OH
**3** R = H



**2** R = OH



**4** R = β-L-Galactopyranosyl

($d$, $J$ = 8.4 Hz, H−C(5′)); 7.62 ($d$, $J$ = 8.4 Hz, H−C(6′))][1]), together with signals at $\delta$(H) 7.53 ($s$, H−C(2′)), 6.48 ($s$, H−C(6)), and 6.70 ($s$, H−C(3)). Comparison of the $^{13}$C-NMR data of **1** with those of isoswertiajaponin [9] led to the conclusion that **1** was a derivative of the latter, with an additional substituent at the glucosyl (Glc) moiety. This substituent was demonstrated to be a 2-methylbutanoyl group by analysis of 1D- and 2D-NMR spectra. The carbonyl C(1‴)-atom at $\delta$(C) 174.5 was connected with the Glc C(2″)-atom at $\delta$(C) 71.5 through an O-atom, based on the HMBC correlation between H−C(2″) ($\delta$(H) 5.37 ($t$, $J$ = 9.5 Hz)) and C(1‴) (*Figure*).

The Glc moiety in **1** was attached to the flavonoid skeleton at C(8), as inferred from the chemical shifts of C(1″) ($\delta$(C) 70.7) and C(8) (103.2), the coupling constant for H−C(1″) ($\delta$(H) 4.88 ($d$, $J$ = 10.2 Hz)), and the HMBC correlation between H−C(1″) and C(7) or C(9) at $\delta$(C) 162.7 and 155.6, respectively. The complete structure of compound **1** was determined by examination of its NMR spectroscopic data including $^{1}$H- and $^{13}$C-NMR, DEPT, $^{1}$H,$^{1}$H-COSY, HMQC, and HMBC experiments. On the basis of the above evidence, **1** was identified as (1*S*)-1,5-anhydro-1-[2-(3,4-dihydroxyphenyl)-5-hydroxy-7-methoxy-4-oxo-4*H*-[1]benzopyran-8-yl]-2-*O*-(2-methylbutanoyl)-D-glucitol, and named *trollisin I*.

Compound **2** was found to be an analogue of **1**. It had the molecular formula $C_{31}H_{30}O_{14}$, as established by negative HR-ESI-MS. The major difference between **2** and **1** was that the Glc substituent at C(2″) ($\delta$(C) 72.8) was a 3,4-dimethoxybenzoyl group in **2** instead of the 2-methylbutyryl moiety in **1**. This substituent was confirmed by 1D- and 2D-NMR experiments, including $^{1}$H- and $^{13}$C-NMR, DEPT, $^{1}$H,$^{1}$H-COSY, HMQC and HMBC (*Table 1*). Atom C(1‴) at $\delta$(C) 164.7 of the benzoyl group was again connected with C(2″) of the Glc moiety through an O-atom, which was supported by the HMBC correlation between H−C(2″) ($\delta$(H) 5.51 ($t$, $J$ = 9.5 Hz)) and C(1‴) (*Figure*).

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[1]) Arbitrary atom numbering, for systematic names, see *Exper. Part*.

Table 1. *$^1$H- and $^{13}$C-NMR Data of* **1** *and* **2**. At 500 and 125 MHz, resp., in ($D_6$)DMSO; $\delta$ in ppm, $J$ in Hz. Arbitrary atom numbering.

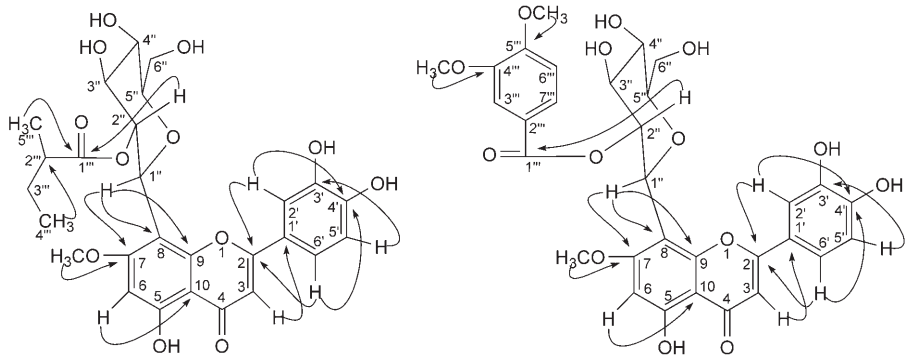
| Position | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) |
| 2 | – | 164.6 | – | 164.5 |
| 3 | 6.70 (*s*) | 102.4 | 6.72 (*s*) | 102.4 |
| 4 | – | 182.1 | – | 182.2 |
| 5 | – | 161.8 | – | 161.6 |
| 6 | 6.48 (*s*) | 94.6 | 6.32 (*s*) | 94.8 |
| 7 | – | 162.7 | – | 162.7 |
| 8 | – | 103.2 | – | 103.4 |
| 9 | – | 155.6 | – | 155.5 |
| 10 | – | 104.2 | – | 104.3 |
| 7-OMe | 3.86 (*s*) | 56.5 | 3.72 (*s*) | 56.7 |
| 1′ | – | 121.7 | – | 121.8 |
| 2′ | 7.53 (*s*) | 114.0 | 7.57 (*s*) | 114.1 |
| 3′ | – | 145.9 | – | 146.0 |
| 4′ | – | 150.1 | – | 150.0 |
| 5′ | 6.89 (*d*, $J$ = 8.4) | 115.7 | 6.91 (*d*, $J$ = 8.5) | 115.8 |
| 6′ | 7.62 (*d*, $J$ = 8.4) | 119.6 | 7.66 (*dd*, $J$ = 8.5, 2.0) | 119.7 |
| 1″ | 4.88 (*d*, $J$ = 10.2) | 70.7 | 5.04 (*d*, $J$ = 10.5) | 70.9 |
| 2″ | 5.37 (*t*, $J$ = 9.5) | 71.5 | 5.51 (*t*, $J$ = 9.5) | 72.8 |
| 3″ | 3.46 – 3.52 (*m*) | 75.7 | 3.46 – 3.51 (*m*) | 75.9 |
| 4″ | 3.48 – 3.53 (*m*) | 70.6 | 3.48 – 3.53 (*m*) | 70.7 |
| 5″ | 3.34 – 3.39 (*m*) | 82.3 | 3.35 – 3.40 (*m*) | 82.4 |
| 6″ | 3.63 – 3.68 (*m*), 3.80 – 3.85 (*m*) | 61.3 | 3.69 – 3.74 (*m*), 3.86 – 3.91 (*m*) | 61.3 |
| 1‴ | – | 174.5 | – | 164.7 |
| 2‴ | 2.06 – 2.12 (*m*) | 40.2 | – | 121.7 |
| 3‴ | 1.25 – 1.31 (*m*) | 25.7 | 7.15 (*d*, $J$ = 2.0) | 111.5 |
| 4‴ | 0.60 (*t*, $J$ = 7.2) | 11.2 | – | 148.2 |
| 5‴ | 0.69 (*d*, $J$ = 6.9) | 16.4 | – | 152.8 |
| 6‴ | – | – | 6.99 (*d*, $J$ = 8.5) | 110.9 |
| 7‴ | – | – | 7.33 (*dd*, $J$ = 8.5, 2.0) | 123.0 |
| 4‴-MeO | – | – | 3.77 (*s*) | 55.5 |
| 5‴-MeO | – | – | 3.78 (*s*) | 55.7 |

Therefore, the structure of **2** was determined as (1*S*)-1,5-anhydro-2-*O*-[(3,4-dimethoxyphenyl)carbonyl]-1-[2-(3,4-dihydroxyphenyl)-5-hydroxy-7-methoxy-4-oxo-4*H*-[1]-benzopyran-8-yl]-D-glucitol, and named *trollisin II*.

The structures of the two known compounds **3** [6] and **4** [7] were determined by comparison of their spectroscopic data with those reported in the literature, including UV, IR, NMR, and MS data.

2. *Antiviral Properties.* All compounds were screened for antiviral activity. Only one constituent, 2″-*O*-(2‴-methylbutyryl)isoswertisin (**3**) showed a moderate *in vitro* antiviral activity against influenza virus A, with an $IC_{50}$ value of 74.3 μg/ml (*Table 2*), in agreement with the therapeutic efficacy of *T. chinensis*.

A preliminary structure – activity-relationship (SAR) study was performed on the basis of *in vitro* antiviral activities. Compounds **1** and **2** both have 3′- and 4′-OH groups



**1** **2**

Figure. *Key HMBC correlations for* **1** *and* **2**

on their *B*-rings, while **3** and **4** only have 4′-OH functions. Further, the structural difference between **3** and **1** is the presence/absence of the 3′-OH group, respectively. Since **1** was not active, in contrast to **3**, an OH function at C(3′) seems to be a disadvantage in terms of antiviral activity. Also, the 7-OH group and the different sizes of **3** and **4** may additionally be limiting factors of antiviral activity.

Table 2. In vitro *Cell Cytotoxicity and Inhibitory Effect of* **3** *against Influenza Virus* (Types A and B). Compounds **1**, **2**, and **4** were not significantly active. MDCK Cells were used for infection (for details, see *Exper. Part*).

| Compound | $CC_{50}$ [μg/ml][a]) | $IC_{50}$ [μg/ml][b]) | | *SI*[c]) | |
|---|---|---|---|---|---|
| | | Type A | Type B | Type A | Type B |
| **3** | 533 | 74.3 | – | 7.17 | – |
| Ribavirin[d]) | > 2000 | 2.2 | 31.4 | > 909 | > 63.7 |

[a]) Concentration for 50% cellular cytotoxicity. [b]) Concentration for 50% virus inhibition. [c]) Selectivity index: $SI = CC_{50}/IC_{50}$. [d]) Positive control.

On the basis of the present work, one can conclude that the major flavonoid constituents in the flowers of *T. chinensis* mainly exist in the form of *C*-glycosides, and these compounds could be a class of active components of the topical flowers.

We would like to thank Dr. *Jian-Nong Li*, Institute of Medicinal Biotechnology, Peking Union Medical College, for his assistance in the antiviral evaluation of the isolated compounds.

## Experimental Part

*General.* Column chromatography (CC): *Sephadex LH-20* (18–110 μm; *Pharmacia*) or *Polyamide* (30–60 or 200 mesh; *Wuxi Electronic Teaching Apparatus Co., Ltd.*, China). Melting points (m.p.) were determined on an *XT-4A* apparatus, and are uncorrected. UV Spectra: *Varian Cary-Eclipse-300* spectrometer, in MeOH soln.; $\lambda_{max}$ in nm. Optical rotations: *AA-10R* polarimeter (*Optical Activity Co., Ltd*). IR Spectra: *Thermo Nicolet Nexus-470* FT-IR spectrometer; in $cm^{-1}$. NMR Spectra: *Bruker DRX-*

*500* NMR spectrometer; $\delta$ in ppm rel. to residual solvent signals, *J* in Hz. HR-ESI-MS: *Bruker APEX-II* mass spectrometer. TOF-ESI-MS: *PE Q-STAR MS/MS* spectrometer; in *m/z*.

*Plant Material*. *Trollius chinensis* BUNGE was purchased in October 2000 from the Chinese crude-drug market in Anguo, Hebei Province, P. R. China, and authenticated by *S.-Q. C.* A voucher specimen (No. 2594) was deposited at the Herbarium of Pharmacognosy, School of Pharmaceutical Sciences, Peking University.

*Extraction and Isolation.* The dried flowers of *T. chinensis* (8 kg) were extracted with 95% EtOH at reflux (3 × 2 h) to afford, after solvent removal *in vacuo*, a crude extract (1.6 kg), which was taken up in $H_2O$, and excessively extracted with petroleum ether (PE), AcOEt, and BuOH. The AcOEt-soluble part (205 g) was purified by CC (*Polyamide* (30–60 mesh); $H_2O$/EtOH 100:0, 80:20, 50:50, 30:70, 5:95): eight fractions (*Fr. A–H*). *Fr. E* (303 mg; eluted with $H_2O$/EtOH 50:50) was further purified by CC (*Sephadex LH-20*; $H_2O$/MeOH 70:30): compound **1** (25 mg). *Fr. F* (eluted with $H_2O$/EtOH 50:50) was subjected to CC (*Sephadex LH-20*, $H_2O$/MeOH 70:30): *Fr. F.1–F.4*. Subfraction *Fr. F.3* (53 mg) was purified by repeated CC (*Sephadex LH-20*; $H_2O$/MeOH 70:30): compound **2** (18 mg). The BuOH-soluble part (150 g) of the above extract was subjected to CC (macroporous resin $D_{101}$; $H_2O$/EtOH 100:0, 70:30, 40:60, 5:95). The fraction eluted with 30% aq. EtOH was concentrated to dryness, and the residue (22 g) was further subjected to CC (*Sephadex LH-20*; $H_2O$/MeOH 90:10 → 40:60): four fractions (*Fr. I–L*). *Fr. K* (59 mg; eluted with 40% aq. MeOH) was purified by repeated CC (*Sephadex LH-20*; MeOH): compounds **3** (35 mg) and **4** (23 mg).

*Trollisin I* (= *(1S)-1,5-Anhydro-1-[2-(3,4-dihydroxyphenyl)-5-hydroxy-7-methoxy-4-oxo-4*H*-[1]benzopyran-8-yl]-2*-O-*(2-methylbutanoyl)*-D-*glucitol*; **1**). Yellow powder. M.p. 152–154°. UV (MeOH): 269, 346. $[\alpha]_D^{20} = -174$ ($c = 0.67$, MeOH). IR (KBr): 3301, 2925, 2854, 1740, 1639, 1606, 1549, 1463, 1264, 1173, 762, 690, 582. $^1$H- and $^{13}$C-NMR: see *Table 1*. ESI-TOF-MS (neg.): 545 ($[M-1]^-$). HR-ESI-MS (neg.): 545.1666 ($[M-1]^-$, $C_{27}H_{29}O_{12}^-$; calc. 545.1659).

*Trollisin II* (= *(1S)-1,5-Anhydro-2-*O*-[(3,4-dimethoxyphenyl)carbonyl]-1-[2-(3,4-dihydroxyphenyl)-5-hydroxy-7-methoxy-4-oxo-4*H*-[1]benzopyran-8-yl]-D-glucitol*; **2**). Yellow powder. M.p. 157–158°. UV (MeOH): 267, 298, 342. $[\alpha]_D^{20} = +83.3$ ($c = 2.4$, MeOH). IR (KBr): 3406, 2924, 2853, 1719, 1655, 1603, 1513, 1445, 1271, 1176, 1081, 838, 572. ESI-TOF-MS (neg.): 625 ($[M-1]^-$). HR-ESI-MS (neg.): 625.1567 ($[M-1]^-$, $C_{31}H_{29}O_{14}^-$; calc. 625.1557).

*Antiviral* in vitro *Assay. Madin–Darby* canine-kidney (MDCK) cells were obtained from the Institute of Virology, Chinese Academy of Preventive Medicine, Beijing, P. R. China. The cells were grown in *Eagle*'s *Minimum Essential Medicine* (*MEM*) medium containing 10% of heat-inactivated fetal bovine serum (FBS) and antibiotics (200 μ/ml penicillin and 200 μ/ml streptomycin). Influenza viruses A (JF 190-15) and B (JF 197-13) were from the Institute of Virology, Chinese Academy of Preventive Medicine. Monolayers of the MDCK cells at *ca*. 85% confluence were infected with the viruses at a multiplicity of infection (MOI) of 0.05 PFU/cell. After 2 h of adsorption at 37°, the cells were washed with phosphate-buffered saline (*PBS*; pH 7.4), and incubated at 37° in the maintenance medium (*MEM* containing 0.1% BSA and 0.005% trypsin) with or without test compound or ribavirin (pos. control) at different concentrations. The cells were collected when the cytopathic effect (CPE) was visually evident in the viral control wells (lacking test compound), which was the case usually after 36–48 h after inoculation. The cytopathic effect was monitored under a light microscope. The drug concentration required for 50% virus inhibition ($IC_{50}$) was derived by linear-regression analysis of a half-log dilution series covering the effective concentrations of the tested drug. Cytotoxicity evaluations were conducted in parallel with the antiviral assays by the CPE method. The selective index (*SI*) was calculated as the ratio $CC_{50}/IC_{50}$.

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