An 18-Norspirostanol Saponin with Inhibitory Action against COX-2 Production from the Underground Part of Trillium tschonoskii

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The genus Trillium (Trilliaceae) contains about 48 related species in temperate eastern Asia and eastern North America, as well as western North America. The majority of Trillium species are associated with the ancient Arcto-Tertiary deciduous forests, which have persisted with dramatic changes in geographical ranges since the early Tertiary period in the northern hemisphere but especially in the North American continent during the Pleistocene Ice Age. Today, each species of Trillium is restricted to one of three geographical areas: eastern Asia, western and eastern North America. Trillium tschonoskii Maxim. grows in Shennongjia Forest District of central China, and its dried underground parts were used as a folk medicine to activate blood, to remove carbuncles, and to ameliorate pains, etc. This medicine was also used as an anti-inflammatory agent in the folk of Zhejiang and Shanxi districts of China. Although several novel steroidal saponins were isolated from its fresh aerial and underground parts, their bioactivities were little revealed before and most studies on this species of plants were carried out twenty years ago. In the course of our studies on the native folk medicines of Shennongjia Forest District, a new 18-nor steroidal saponin was obtained from the water soluble part of Trillium tschonoskii rhizomes. This paper deals with the structural elucidation of a novel saponin and a known compound isolated from this plant, and their inhibitory effect on COX-2 production was observed.

1 was obtained as a colorless powder from water-soluble part, [α]D20 = 45° (c=0.65, 15% acetonitrile). Its IR spectrum showed absorption maxima at 3431 cm−1, 1690 cm−1, and 1649 cm−1, due to a hydroxyl and an α,β-unsaturated ketone system, respectively. Its HR-FAB-MS showed a quasi-molecular ion peak at m/z 1057.4140 [M+Na]+, which indicated a formula C45H62O14Na (Caled: 1057.4104). Positive coloration reactions for Molish and Liebermann–Buchard tests indicated a steroidal saponin skeleton for 1. The 1H-NMR spectrum of 1 showed one singlet methyl signal at δ 1.23, one doublet methyl signal at δ 0.998 (d, J=6.5 Hz) and one olefinic signal at δ 6.00 (d, J=5.5 Hz), ascribable to a steroidal sapogenin moiety. The 13C-NMR spectrum of 1 showed 27 carbon signals, including one carbonyl carbon signal at δ 204.50, four olefinic carbon signals at δ 177.46, 141.97, 137.39, 128.29, one acetal carbon signal at δ 114.55, two oxygenated methane carbon signals at δ 64.98, 61.56, and six oxygenated methine carbon signals at δ 84.11, 81.49, 75.37, 74.61, 67.72, 61.88. In addition to signals attributable to a steroidal aglycone, four anomeric proton signals were observed in the 1H-NMR spectrum of 1 at δ 6.39 (1H, s), 6.22 (1H, d, J=2.4 Hz), 4.97 (1H, d, J=7.6 Hz), 4.46 (1H, d, J=7.5 Hz), and four anomeric carbon signals in the 13C-NMR spectrum of 1 at δ 101.38, 111.72, 106.62, 100.86. Upon acid hydrolysis of 1 with 2.0 mol HCl, rhamnose, apiose, xylose and arabinose were detected in the supernatant on the paper chromatography and TLC. The proton and carbon resonances due to sugar moieties (Table 1) are identical with those of corresponding sugar moieties in trillenoside A. In comparison of carbon signals due to aglycone of 1 with those of trillenoside A, downfield shifts of 3.3 ppm for C-6, 2.15 ppm for C-7 and 0.28 ppm for C-8 were observed in the 13C-NMR spectrum of 1, respectively. The downfield shifts of 0.41 ppm for H-6, 2.15 ppm for H-7b and 0.28 ppm for H-8 were also observed in the 13C-NMR spectrum of 1, respectively. These showed that a hydroxy group was substituted at C-7 of aglycone in 1, which was consistent with the HMBC results of 1. The correlations of H-7 at δ 5.40 (1H, br s) and H-8 at δ 2.49 (1H, br d, J=4.9 Hz), H-7 at δ 5.40 (1H, br s) and H-6 at δ 6.00 (d, J=5.5 Hz) in the NOESY spectrum of 1, indicated a β-orientation of hydroxy group at C-7, which was agreeable with the couple constants between H-7 and H-8, as well as H-7 and H-6. Therefore, 1 was identified as 7β-hydroxy trillenogenin-1-O-D-apiofuranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside.

The inhibitory actions of 1 and 2 against COX-2 production were observed in macrophagocytes of the mouse abdominal cavity stimulated by LPS at 10 μg/ml. The activities of 9.7×10−4 mol/l of 1 and 9.8×10−4 mol/l of 2 were approximately equivalent to that of 1.1×10−4 mol/l of aspirin (Table 2).

Experimental

Optical rotations were recorded with a Perkin-Elmer 241 spectropolarimeter. IR spectra were measured on a Nicolet FT360 instrument as samples in pressed KBr disks. 1D and 2D NMR spectra were recorded using Bruker...
The rhizomes of *Trillium teshonoki* were purchased at Muyu, a town of Shennongjia Forest District of China in May 2005 and identified by Professor Chen Faju. A voucher specimen (Herbarium No.: 2005ZW03128) has been deposited in the Herbarium of Department of Medicinal Plants, College of Chemistry and Life Science, China Three Gorges University, Yichang.

**Extraction and Isolation** Air-dried powdered rhizomes (6.4 kg) was extracted with methanol under reflux. After the removal of solvent in vacuo and freeze-drying, the methanol extract (2427 g) was obtained. The extract was suspended in water (2.2 L), and then extracted with CHCl₃, EtOAc and n-BuOH successively. The rest solution was reduced in vacuo to a small volume (1.5 L). The methanol extract (2427 g) was subjected to macroporous resin column chromatography in elution with gradient solvent system (100% water→10% EtOH). The extract was refluxed with 2.0M HCl, and then subjected to repeated Sephadex LH-20 column chromatography in elution with water, acetonitrile, and 30% acetonitrile. The 13% and 15% acetonitrile eluates were further separated by repeated Sephadex LH-20 column chromatography in elution with water, acetonitrile. The 13% and 15% acetonitrile eluates were further separated by repeated Sephadex LH-20 column chromatography in elution with water, acetonitrile. The 13% and 15% acetonitrile eluates were further separated by repeated Sephadex LH-20 column chromatography in elution with water, acetonitrile.

**Bioassay** 1.0 ml of 1% soluble starch for each mouse was injected into the abdominal cavity of C57BL/6 mice. Once the mice were decapitated after 3 d, 8.0 ml of Hanker’s solution for each mouse was injected into their abdominal cavities to obtain the cell solution under aseptic condition. The cell solution was centrifugated for 8 min at 1000 rpm, and the precipitates were washed away with Hanker’s solution and the culture medium without calf serum was added. After 12 h, the culture solution was taken away and the LPS solution was added to the plates, making LPS concentration be 1.0 μg/ml. After they were incubated for 6 h, samples were added and cultivated for 1 h according to the groups list in Table 2. The hydrolysate was neutralized with NaHCO₃, and then extracted with chloroform. The chloroform-insoluble part was detected on PC.
and TLC according to the procedures.17

**Compound 1:** A colorless powder, [α]$_D^{20}$ = -45° (c=0.65, 15% acetonitrile). HR-FAB-MS (positive mode) m/z: 1057.4110 [M+Na]$^+$ (Calcd for C$_{47}$H$_{70}$O$_{25}$Na: 1057.4104). ESI-MS (positive ion mode) m/z: 1073 [M+K]$^+$, 1057 [M+Na]$^+$, (negative ion mode) m/z: 1033 [M−H]$^-$, 901 [M−api−H]$^-$, 769 [M−api−xyl−H]$^-$, 3430, 2922, 1690, 1649, 1050 cm$^{-1}$. $^1$H-NMR (500 MHz, pyridine-d$_5$) and $^{13}$C-NMR (125 MHz, pyridine-d$_5$) see Table 1.

**Compound 2:** A white powder, [α]$_D^{20}$ = -78° (c=2.5, 15% acetonitrile). $^1$H- and $^{13}$C-NMR data were well agreeable with those of trillenoside A.16

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**References**