Three New Sesquiterpene Glycosides from the Rhizomes of *Trillium tschonoskii*

Jie Yang 1,2, Yin-Jun Yang 2,3, Xin-Guang Sun 2, Jie Zhang 2, Yang Zhao 2, Bei Wang 1,2, Qian-Zhi Ding 2, Bao-Lin Guo 3 and Bai-Ping Ma 1,2,*

1 School of Traditional Chinese Medicine, Guangdong Pharmaceutical University, Guangzhou 510006, China; yangjie0528@163.com (J.Y.); wbshell125@126.com (B.W.)
2 Beijing Institute of Radiation Medicine, No. 27, Taiping Road, Beijing 100850, China; iyangyinjun@163.com (Y.-J.Y.); sxgzhwu07@163.com (X.-G.S.); zhangjie061003@163.com (J.Z.); mmyzhao@163.com (Y.Z.); dqz1990@163.com (Q.-Z.D.)
3 Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing 100193, China; guobaolin010@163.com

* Correspondence: mabaiping@sina.com; Tel.: +86-10-6821-0077 (ext. 930265)

Received: 24 June 2017; Accepted: 1 August 2017; Published: 2 August 2017

Abstract: Three new sesquiterpene glycosides, possessing a rare aglycone with a sulfonyl between C-1 and C-15 positions, named 3-(3′E-7′R,8′-dihydroxy-4′,8′-dimethyl-3′-nonenyl)-2,5-dihydro-1,1-dioxo-thiophen 7′-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranoside (1), 3-(3′E-7′R,8′-dihydroxy-4′,8′-dimethyl-3′-nonenyl)-2,5-dihydro-1,1-dioxothiophen 7′-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranoside (2), and 3-(3′E-7′R,8′-dihydroxy-4′,8′-dimethyl-3′-nonenyl)-2,5-dihydro-1,1-dioxothiophen 7′-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-6′-O-acetyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranoside (3), respectively, were isolated from the rhizomes of *Trillium tschonoskii*. Their structures were established on the basis of spectroscopic data, including HR-ESI-MS, IR, 1D and 2D NMR. The cytotoxic properties of the three compounds were investigated using human hepatic L02 cells.

Keywords: *Trillium tschonoskii*; sesquiterpene glycosides; separation

1. Introduction

*Trillium tschonoskii* Maxim is perennial herb, mainly distributed in Hubei, Shanxi and Anhui provinces of China at an altitude of 1600–3200 m [1]. The rhizomes of *T. tschonoskii* named Yan Ling Cao, have been used as a traditional Chinese medicine (TMC) for the treatment of headache, traumatic injury, and neurasthenia [2]. Recent pharmacological studies have shown that Yan Ling Cao possesses anti-tumor, anti-inflammatory, analgesic and blood coagulation activities [3–7]. Previous phytochemical studies on the *Trillium* reveals that steroidal saponins constitute the main chemical components [6,8–12]. Besides, it also contains a few phenylpropanoid glycosides [13] and sesquiterpenoid glycosides [3,13,14]. In this study, a chemical investigation on *T. tschonoskii* led to the isolation of three new sesquiterpene glycosides that have a rare aglycone with a sulfonyl between C-1 and C-15 positions. Their structures were identified using the spectroscopic techniques of HR-ESIMS, IR, and NMR. The cytotoxic activity of the three compounds were evaluated against L02 cells.

2. Results and Discussion

The rhizomes of *T. tschonoskii* were extracted using 50% aq. EtOH. The extract was subjected to macroporous resin SP825 column chromatography to afford five fractions (Fr. A–Fr. E). Fraction C was subsequently separated on silica-gel, MCI, ODS, preparative and semi-preparative HPLC to provide three new sesquiterpene glycosides (Figure 1), named 3-(3′E-7′R,8′-dihydroxy-4′,8′-dimethyl-
3′-nonenyl)-2,5-dihydro-1,1-dioxo-thiophen 7′-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl (1, 2, 3) was a dimeric compound containing two identical monomers. The 1H-NMR spectrum of 1 (Table 1) showed three tertiary methyl group signals (δH 1.37, 1.32, 1.61), two olefinic protons (δH 4.89 (d, J = 7.2 Hz), 5.12 (d, J = 7.8 Hz)), as well as signals for three anomeric protons at (δH 4.89 (d, J = 7.9 Hz), 5.12 (d, J = 7.8 Hz), and 5.15 (d, J = 7.8 Hz)). The 13C combined with HSQC NMR spectra of 1 indicated a structure with a total of 33 C-atom signals. Four of them were attributed to the aglycone carbons including four olefinic carbons (δC 171.9), two sulfonated methylene carbons (δC 57.4, 58.0), and three tertiary methyl carbons (δC 16.1, 25.3, 26.8), while the remaining carbon signals were characteristic to three glycosyl moieties. By comparing the NMR data of 1 with 3-(3′E,7′,8′-dihydroxy-4′,8′-dimethyl-3′-nonenyl)-2,5-dihydro-1,1-dioxo-thiophen [15], the structure of 1 was similar to 3-(3′E,7′,8′-dihydroxy-4′,8′-dimethyl-3′-nonenyl)-2,5-dihydro-1,1-dioxo-thiophen except for one sugar moiety at C-10 of 1, which was further supported by 1H−1H COSY correlation (Figure 2) of H-1/H-2, H-5/H-4 and H-6, H-9/H-8 and H-10, and HMBC correlation of H-2/C-3, C-4 and C-15, H-6/C-8 and C-14, H-10/C-11 and C-12, H-12/C-10, C-11 and C-13. Comparison of the NMR data of 1 with [2,3-S-trans,10R,6E]-7,11-dimethyl-3-methylene-1,6-dodecadien-10,11-diol 10-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl (1, 2, 3) suggested that they shared the same sugar chain. The sugar moiety was further assigned by HSQC, HMBC and 1H−1H COSY experiments. Furthermore, the HMBC correlations between H-1-Glc′ (δ 4.89) and C-10 (δ 90.0), H-1-Glc” (δ 5.12) and C-4-Glc′′ (δ 80.9), H-1-Glc′ (δ 5.15) and C-4-Glc′′ (δ 80.9) (Figure 2) verified the linkage of the sugar unit and its location at C-10 of 1. The absolute configuration at C-10 of 1 was confirmed as R by the values of glycosylation shift.

**Figure 1.** The chemical structures of compounds 1–3.
of α-β-(pro-S side), and β-β-(pro-R side) carbons of secondary alcohols to which glucosyl moieties were attached [16]. Furthermore, the 13C chemical shifts at C-8 and C-12 of 1 were quite similar to those of (2, 3-trans, 10R, 6E)-7, 11-dimethyl-3-methylene-1, 6-dodecadien-10, 11-diol 10-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranoside (10R) [14], 7, 11-dimethyl-3-methylene-1, 6-dodecadien-10, 11-diol 10-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranoside (10R) [13], and icariside C4 (10R) [17], while different from those of icariside C1 [17]. The assignments at C-8 and C-9 of icariside C4 and icariside C1 in the literature [17] should be interchanged. Based on the above evidence, 1 was defined as 3-(3′E-7′R, 8′-dihydroxy-4′, 8′-dimethyl-3′-nonenyl)-2, 5-dihydro-1, 1-dioxo-thiophen 7′-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranoside.

![Figure 2. Key HMBC (arrows) and 1H−1H COSY (thick lines) correlations of compounds 1–3.](image)

Compound 2 was obtained as a white amorphous powder and its molecular formula C27H36O14S was established by HR-ESIMS at m/z 625.2515 [M – H]− (calcd. for C27H46O13S 625.2530). Comparing the NMR and MS data of 2 with 1, it was determined that 2 had the same aglycone as 1. The 13C-NMR resonances of the sugar unit were identified by HSQC and further confirmed by HMBC experiments. The sugar moieties of 2 were the same as those of 7, 11-dimethyl-3-methylene-1, 6-dodecadien-10, 11-diol 10-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranoside [13], as revealed by comparing the NMR data. The HMBC spectrum showed long-range correlations between H-1-Glc′ (δ 8.91) and C-10 (δ 90.0), H-1-Glc" (δ 5.18) and C-4-Glc′ (δ 81.3) (Figure 2), which assigned the linkage of the sugar moiety. Therefore, 2 was defined as 3-(3′E-7′R, 8′-dihydroxy-4′, 8′-dimethyl-3′-nonenyl)-2, 5-dihydro-1, 1-dioxo-thiophen 7′-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranoside.

Compound 3 was obtained as a white amorphous powder and the molecular formula C35H50O20S was deduced by HR-ESIMS at m/z 829.3126 [M – H]− (calcd. for C35H50O20S 829.3164). The NMR data of 3 were very similar to 1, except for the presence of the CH3CO group. Furthermore, the CH3CO group located at the OH group of C-6 position in the inner Glc′, which turned into ester, was supported by the HMBC correlations between H-6-Glc′ (δ 5.13, 4.77) and C-CH3CO (δ 170.8). The sugar moieties of 3 were further assigned by the HSQC, HMBC, and 1H−1H COSY experiments. Therefore,
3 was defined as 3-(3′E,7′R,8′-dihydroxy-4′,8′-dimethyl-3′-nonenyl)-2,5-dihydro-1,1-dioxo-thiophen 7′-O-β-D-glucopyranosyl-6′-O-acetyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranoside.

Table 1. 1H-NMR, 13C-NMR data for compounds 1-3.

| No. | δC | δH | δC | δH | δC | δH |
|-----|----|----|----|----|----|----|
| 2   | 58.0 | 3.85 (2H, m) | 58.0 | 3.85 (2H, m) | 58.0 | 3.85 (2H, m) |
| 3   | 138.7 | 76.5 | 138.7 | 76.5 | 138.7 | 76.5 |
| 4   | 57.4 | 2.89 (2H, m) | 57.4 | 2.89 (2H, m) | 57.4 | 2.89 (2H, m) |
| 5   | 33.1 | 2.07 (2H, m) | 33.1 | 2.07 (2H, m) | 33.1 | 2.07 (2H, m) |
| 2′  | 25.6 | 2.09 (2H, m) | 25.6 | 2.09 (2H, m) | 25.6 | 2.09 (2H, m) |
| 3′  | 123.6 | 5.34 (1H, br t, J = 7.2Hz) | 123.6 | 5.34 (1H, br t, J = 7.2Hz) | 123.6 | 5.34 (1H, br t, J = 7.2Hz) |
| 4′  | 136.8 | 76.8 | 136.8 | 76.8 | 136.8 | 76.8 |
| 5′  | 36.2 | 2.74 (1H, m) | 36.2 | 2.74 (1H, m) | 36.2 | 2.74 (1H, m) |
| 6′  | 30.8 | 1.84 (1H, m) | 30.8 | 1.84 (1H, m) | 30.8 | 1.84 (1H, m) |
| 7′  | 90.0 | 3.75 (1H, dd, J = 1.5, 9.5Hz) | 90.0 | 3.75 (1H, dd, J = 1.5, 9.5Hz) | 90.0 | 3.75 (1H, dd, J = 1.5, 9.5Hz) |
| 8′  | 71.9 | 71.9 | 71.9 | 71.9 | 71.9 | 71.9 |
| 9′  | 25.3 | 1.37 (3H, s) | 25.3 | 1.37 (3H, s) | 25.3 | 1.37 (3H, s) |
| 8′-CH3 | 26.8 | 1.32 (3H, s) | 26.8 | 1.32 (3H, s) | 26.8 | 1.32 (3H, s) |
| 4′-CH3 | 61.9 | 4.47 (1H, o) | 61.9 | 4.47 (1H, o) | 61.9 | 4.47 (1H, o) |
| Glc-1′ | 105.7 | 4.89 (1H, d, J = 7.8 Hz) | 105.7 | 4.89 (1H, d, J = 7.8 Hz) | 105.7 | 4.89 (1H, d, J = 7.8 Hz) |
| Glc-2′ | 74.3 | 4.05 (1H, o) | 74.3 | 4.05 (1H, o) | 74.3 | 4.05 (1H, o) |
| Glc-3′ | 76.5 | 4.22 (1H, o) | 76.5 | 4.22 (1H, o) | 76.5 | 4.22 (1H, o) |
| Glc-4′ | 80.9 | 4.28 (1H, o) | 80.9 | 4.28 (1H, o) | 80.9 | 4.28 (1H, o) |
| Glc-5′ | 76.4 | 3.95 (1H, o) | 76.4 | 3.95 (1H, o) | 76.4 | 3.95 (1H, o) |
| Glc-6′ | 61.9 | 4.47 (1H, o) | 61.9 | 4.47 (1H, o) | 61.9 | 4.47 (1H, o) |
| Glc-1′′ | 105.0 | 5.12 (1H, d, J = 7.8 Hz) | 105.0 | 5.12 (1H, d, J = 7.8 Hz) | 105.0 | 5.12 (1H, d, J = 7.8 Hz) |
| Glc-2′′ | 74.7 | 4.05 (1H, o) | 74.7 | 4.05 (1H, o) | 74.7 | 4.05 (1H, o) |
| Glc-3′′ | 76.7 | 4.22 (1H, o) | 76.7 | 4.22 (1H, o) | 76.7 | 4.22 (1H, o) |
| Glc-4′′ | 80.9 | 4.28 (1H, o) | 80.9 | 4.28 (1H, o) | 80.9 | 4.28 (1H, o) |
| Glc-5′′ | 78.5 | 3.95 (1H, o) | 78.5 | 3.95 (1H, o) | 78.5 | 3.95 (1H, o) |
| Glc-6′′ | 61.8 | 4.49 (1H, o) | 61.8 | 4.49 (1H, o) | 61.8 | 4.49 (1H, o) |
| Glc-1′′′ | 104.5 | 5.15 (1H, d, J = 7.8 Hz) | 104.5 | 5.15 (1H, d, J = 7.8 Hz) | 104.5 | 5.15 (1H, d, J = 7.8 Hz) |
| Glc-2′′′ | 75.0 | 4.05 (1H, o) | 75.0 | 4.05 (1H, o) | 75.0 | 4.05 (1H, o) |
| Glc-3′′′ | 76.6 | 4.17 (1H, o) | 76.6 | 4.17 (1H, o) | 76.6 | 4.17 (1H, o) |
| Glc-4′′′ | 71.5 | 4.17 (1H, o) | 71.5 | 4.17 (1H, o) | 71.5 | 4.17 (1H, o) |
| Glc-5′′′ | 78.2 | 3.95 (1H, o) | 78.2 | 3.95 (1H, o) | 78.2 | 3.95 (1H, o) |
| Glc-6′′′ | 62.5 | 4.52 (1H, d, J = 2.4, 11.4 Hz) | 62.5 | 4.52 (1H, d, J = 2.4, 11.4 Hz) | 62.5 | 4.52 (1H, d, J = 2.4, 11.4 Hz) |
| CH3CO | 20.8 | 2.08 (3H, s) | 20.8 | 2.08 (3H, s) | 20.8 | 2.08 (3H, s) |

δ in CD3OD, in ppm from tetramethylsilane (TMS). 1H-NMR at 600 MHz, 13C-NMR at 150 MHz; o: overlapped with other signals; m: multiplet signals.

Compound 1–3 were evaluated for cytotoxicity against L02 cells. All three compounds showed no cytotoxic activity at 100 μM.

3. Experimental

3.1. General Experimental Procedures

IR spectra, HR-ESIMS and NMR were recorded on a VERTEX 70 FT Infrared Spectrometer, Synapt MS (Waters Corporation, Milford, MA, USA) and Varian UNITYINOVA 600 spectrometer (600 MHz for 1H-NMR and 150 MHz for 13C-NMR, Palo Alto, CA, USA) in pyridine-d5 (Sigma-Aldrich, St. Louis, MO, USA), respectively. HPLC analyses were performed on Agilent 1100 system (Agilent Technologies, Santa Clara, CA, USA) equipped with a Silgreen C18 column (4.6 mm × 250 mm, ODS, 5 μm, Silgreen Co. Ltd., Beijing, China) and an Alltech 2000 evaporative light scattering detector (Temperature: 110 °C, Gas: 2.4 L/min, Alltech Corporation, Deerfield, IL, USA). Preparative HPLC was performed on an NP7000 module (Hanbon Co. Ltd., Huaian, China) equipped with a Shodex RID 102 detector (Showa Denko Group, Tokyo, Japan), and a Silgreen C18 column (20.0 mm × 250 mm, ODS, 5 μm,
Silgreen Co. Ltd., Beijing, China). Semi-preparative HPLC was performed on a Waters 515 pump (Waters Corporation, Milford, MA, USA) equipped with a Shodex RID 101 detector (Showa Denko Group, Tokyo, Japan), using a Silgreen C18 column (10.0 mm × 250 mm, ODS, 5 µm, Silgreen Co. Ltd., Beijing, China). TLC was performed on silica gel GF254 plates (Qingdao Marine Chemical, Qingdao, China). Macroporous resin SP825 (Mitsubishi Chemicals, Tokyo, Japan), silica gel H (Qingdao Marine Chemical, Qingdao, China), and MCI gel (50 µm, Mitsubishi Chemicals, Tokyo, Japan) were applied to the performance of column chromatography.

3.2. Plant Material

The rhizomes of *T. tschonoskii* were collected from the Shennongjia of Hubei province, were identified by Professor Bao-Lin Guo (Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences). A voucher specimen (No. 151010) was deposited in the author’s laboratory in the Beijing Institute of Radiation Medicine.

3.3. Extraction and Isolation

The rhizomes of *T. tschonoskii* (5 kg) were crushed and extracted with 50% aq. EtOH at reflux three times (40 L, 30 L, and 30 L each for 2 h). The filtered solution was concentrated in vacuo. The supernatants were applied to a macroporous resin SP825 column, eluted with EtOH/H_{2}O (5:95, 30:70, 50:50, 75:25 and 95:10, v/v) to yield five fractions (Fr. A–Fr. E). Fr. C (120 g) was subjected to silica-gel CC with a gradient mixture of CHCl_{3}:MeOH:H_{2}O (5:1:0.01, 65:25:4, and 2:1:0.01) as the eluent, and five subfractions were obtained (Fr. C-1–Fr. C-5). Fr. C-3 (35 g) was further subjected to MCI gel CC with a gradient mixture of acetone/H_{2}O (10:90, 15:85, 20:80, 30:70 and 50:50, v/v) as the eluent. As a result, a total of 30 fractions were collected (Fr. C-3-1–Fr. C-3-30). Fr. C-3-6 was purified by preparative HPLC with ACN/H_{2}O (20:80, v/v) to obtain seven fractions (Fr. C-3-6-1–Fr. C-3-6-7). Fr. C-3-6-4 underwent semi-preparative HPLC with acetone/H_{2}O (25:75, v/v) to yield 1 (55 mg) and 2 (12 mg). Fr. C-3-12 was purified by preparative HPLC with ACN/H_{2}O (22:78, v/v) to yield five fractions (Fr. C-3-12-1–Fr. C-3-12-5). Fr. C-1-12-5 was purified by semi-preparative HPLC with ACN/H_{2}O (20:80, v/v) to yield 3 (15 mg).

3-(3′E)-7′R,8′R-dihydroxy-4′,8′-dimethyl-3′-nonenyl)-2,5-dihydro-1,1-dioxo-thiophen 7′-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranoside (1): White amorphous power; [α]_{D}^{20} = −10.5^\circ\text{ (c 0.085, MeOH)}; IR (KBr) ν_{max} 3408, 2970, 2927, 1642, 1382, 1306, 1236, 1159, 1072, 1026; 1H-NMR and 13C-NMR spectroscopic data, see Table 1; HR-ESI-MS (positive) m/z 789.3241 [M + H]^+ (calcd. for C_{33}H_{35}O_{19}S 789.3215), 811.3036 [M + Na]^+, 627.2696 [M + H − Glc]^+, 465.2148 [M + H − Glc − Glc]^+, 303.1644 [M + H − Glc − Glc − Glc]^+. 3-(3′E)-7′R,8′R-dihydroxy-4′,8′-dimethyl-3′-nonenyl)-2,5-dihydro-1,1-dioxo-thiophen 7′-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranoside (2): White amorphous powder; [α]_{D}^{20} = −10.3^\circ\text{ (c 0.082, MeOH)}; IR (KBr) ν_{max} 3433, 2928, 1642, 1383, 1306, 1237, 1074, 1041; 1H-NMR and 13C-NMR spectroscopic data, see Table 1; HR-ESIMS (positive) m/z 627.2679 [M + H]^+ (calcd. for C_{27}H_{47}O_{14}S 627.2687), 649.2488 [M + Na]^+, 465.2147 [M + H − Glc]^+, 303.1637 [M + H − Glc − Glc]^+. 3-(3′E)-7′R,8′R-dihydroxy-4′,8′-dimethyl-3′-nonenyl)-2,5-dihydro-1,1-dioxo-thiophen 7′-O-β-D-glucopyranosyl-6′-O-acetyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranoside (3): White amorphous powder; [α]_{D}^{20} = −12.5^\circ\text{ (c 0.088, MeOH)}; IR (KBr) ν_{max} 3428, 2970, 2926, 1736, 1644, 1383, 1307, 1239, 1161, 1072, 1029; 1H-NMR and 13C-NMR spectroscopic data, see Table 1; HR-ESIMS (positive) m/z 831.3329 [M + H]^+ (calcd. for C_{35}H_{35}O_{20}S 831.3320), 853.3165 [M + Na]^+, 669.2820 [M + H − Glc]^+, 507.2261 [M + H − Glc − Glc]^+, 303.1612 [M + H − Glc − Glc − (Ac-Glc)]^+.
3.4. Acid Hydrolysis and GC-MS Analysis

Compound 1 (1.4 mg), 2 (1.6 mg), and 3 (1 mg) were hydrolyzed with 2 N aq. CF₃COOH (5 mL) for 5 h at 95 °C, respectively. After extraction with CH₂Cl₂ (3 × 5 mL), the aq. layer was repeatedly evaporated to dryness with EtOH until neutral, and then analyzed by TLC over silica gel (CHCl₃:MeOH:H₂O, 8:5:1) by comparison with authentic samples. Furthermore, the residue of sugars was dissolved in anhydrous pyridine (2 mL), and L-cysteine methyl ester hydrochloride (3 mg) was added. The mixture was stirred at 60 °C for 1 h, then 3 mL of HMDS-TMCS (hexamethyldisilazane:trimethylchlorosilane, 2:1) was added, and the mixture was stirred at 60 °C for 30 min. The precipitate was centrifuged off, and the supernatant was analyzed by GC-MS (Agilent Technologies 5977A MSD). The absolute configurations were determined by comparing the retention times with derivatives prepared in a similar way from standard D-glucose (Sigma-Aldrich). Identification of D-glucose was carried out for compounds 1–3, giving two peaks at 3.75 min and 4.15 min which were two silylated derivatives (Supplementary Material, Figure S25).

3.5. Cytotoxicity Assay

The cytotoxic activity was measured by MTT assay [18]. L02 Cells were seeded in 96-well plates and treated 24 h later with various (100 µM, 50 µM, 25 µM, 12.5 µM, 6.25 µM, 3.125 µM) concentrations of compounds 1–3. After 24 h of incubation, MTT was added to all wells. Plates were incubated for another 24 h, and cell viability was measured by observing absorbance at 492 nm.

4. Conclusions

In conclusion, three new sesquiterpene glycosides were isolated from the rhizomes of T. tschonoskii. Their structures were elucidated by extensive analysis of spectroscopic methods including 1D and 2D NMR experiments (HSQC, HMBC, 1H–1H COSY), IR, and HR-ESI-MS. The aglycone of the compounds found in this study was a rare aglycone which contains a sulfonyl between C-1 and C-15 positions. Compounds 1–3 were investigated for their cytotoxic activity against L02 cells, and no obvious cytotoxic activity was found.

Supplementary Materials: The supplementary materials are available online.

Author Contributions: Jie Yang, Yin-Jun Yang, and Xin-Guang Sun performed the isolation and structure elucidation of the constituents. Jie Zhang, Bei Wang, and Qian-Zhi Ding contributed in the interpretation of the spectra and also part of the preparation of the manuscript. Yang Zhao partially contributed the structure elucidation, analyzed the data and together with Jie Yang and Bao-Lin Guo prepared the manuscript. Bai-ping Ma planned, designed and organized the whole research study. All authors approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wu, Z.Y.; Yu, D.J.; Cui, H.B. Flora Republicae Popularis Sinicae; Academia Sinicae: Beijing, China, 1978.
2. Xie, W. The Compilation of Chinese Herbal Medicine; People’s Medical Publishing House: Beijing, China, 2000.
3. Wang, J.; Zou, K.; Zhang, Y.; Liu, C.; Wu, J.; Zhou, Y.; Dan, F.; Zhang, Y. An 18-norspirostanol saponin with inhibitory action against COX-2 production from the underground part of Trillium tschonoskii. Chem. Pharm. Bull. 2007, 55, 679–681. [CrossRef] [PubMed]
4. Wang, H.; Zhai, Z.; Li, N.; Jin, H.; Chen, J.; Yuan, S.; Wang, L.; Zhang, J.; Li, Y.; Yun, J.; et al. Steroidal saponin of Trillium tschonoskii. Reverses multidrug resistance of hepatocellular carcinoma. Phytomedicine 2013, 20, 985–991. [CrossRef] [PubMed]
5. Li, Y.; Liu, C.; Xiao, D.; Han, J.; Yue, Z.; Sun, Y.; Fan, L.; Zhang, F.; Meng, J.; Zhang, R.; et al. Trillium tschonoskii steroidal saponins suppress the growth of colorectal Cancer cells In Vitro and In Vivo. J. Ethnopharmacol. 2015, 168, 136–145. [CrossRef] [PubMed]
6. Meng, C.W.; Peng, C.; Zhou, Q.M.; Yang, H.; Guo, L.; Xiong, L. Spirostanols from the roots and rhizomes of Trillium tschonoskii. Phytochem. Lett. 2015, 14, 134–137. [CrossRef]
7. Yu, L.L.; Zou, K.; Wang, J.Z.; Zhu, L.B.; Zhou, Y.; Yang, J. Study on the anti-inflammatory, analgesic and thrombosis effects of extract of *Trillium tschonoskii* Maxim. *Shizhen J. Tradit. Chin. Med. Res.* 2008, 19, 1178–1180.

8. Wei, J.C.; Man, S.L.; Gao, W.Y.; Chai, X.; Zhao, W.S.; Wang, Y.; Jing, S.S. Steroidal saponins from the rhizomes of *Trillium tschonoskii* Maxim. *Biochem. Syst. Ecol.* 2012, 14, 112–116. [CrossRef] [PubMed]

9. Hayes, P.Y.; Lehmann, R.; Perman, K.; Kitting, W.; De Voss, J.J. Steroidal saponins from the roots of *Trillium erectum* (Beth root). *Phytochemistry* 2009, 70, 105–113. [CrossRef] [PubMed]

10. Shafiqur, R.; Ismail, M.; Shah, M.R.; Adhikari, A.; Anis, I.; Ahmad, M.S.; Khurram, M. Govanoside A, a new steroidal saponin from rhizomes of *Trillium govanianum*. *Steroids* 2015, 104, 270–275. [CrossRef] [PubMed]

11. Yokosuka, A.; Mimaki, Y. Steroidal glycosides from the underground parts of *Trillium erectum* and their cytotoxic activity. *Phytochemistry* 2008, 69, 2724–2730. [CrossRef] [PubMed]

12. Ono, M.; Yanai, Y.; Ikeda, T.; Okawa, M.; Nohara, T. Steroids from the underground parts of *Trillium kamtschaticum*. *Chem. Pharma. Bull.* 2003, 51, 1328–1331. [CrossRef]

13. Ono, M.; Takamura, C.; Sugita, F.; Masuoka, C.; Yoshimitsu, H.; Ikeda, T.; Nohara, T. Two new steroid glycosides and a new sesquiterpenoid glycoside from the underground parts of *Trillium kamtschaticum*. *Chem. Pharm. Bull.* 2007, 55, 551–556. [CrossRef] [PubMed]

14. Chai, J.; Song, X.; Wang, X.; Mei, Q.; Li, Z.; Cui, J.; Tang, Z.; Yue, Z. Two new compounds from the roots and rhizomes of *Trillium tschonoskii*. *Phytochem. Lett.* 2014, 10, 113–117. [CrossRef]

15. Abraham, W.R.; Arfmanna, H.A.; Giersch, W. Microbial hydroxylation of precursors of sinensal. *Z. Für Naturforsch.* C 1992, 47, 851–858.

16. Seo, S.; Tomita, Y.; Tori, K.; Yoshimura, Y. Determination of the absolute configuration of a secondary hydroxy group in a chiral secondary alcohol using glycosidation shifts in carbon-13 nuclear magnetic resonance spectroscopy. *J. Am. Chem. Soc.* 1978, 100, 3331–3339. [CrossRef]

17. Miyase, T.; Ueno, A.; Takizawa, N.; Kobayashi, H.; Oguchi, H. Studies on the glycosides of *Epimedium grandiflorum* MORR. var. thunbergianum (MIQ.) NAKAI. I. *Chem. Pharm. Bull.* 1987, 35, 3713–3719. [CrossRef]

18. Zhang, B.J.; Teng, X.F.; Bao, M.F.; Zhong, X.H.; Ni, L.; Cai, X.H. Cytotoxic indole alkaloids from Tabernaemontana officinalis. *Phytochemistry* 2015, 120, 46–52. [CrossRef] [PubMed]

**Sample Availability:** Samples of the compounds 1–3 are available from the authors.

© 2017 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).