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

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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-dioxo-thiophen 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-dioxo-thiophen 7′-*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-glucopyranoside (**1**), 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 (**2**), 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 (**3**), which were identified by NMR, IR and HR-ESI-MS techniques.

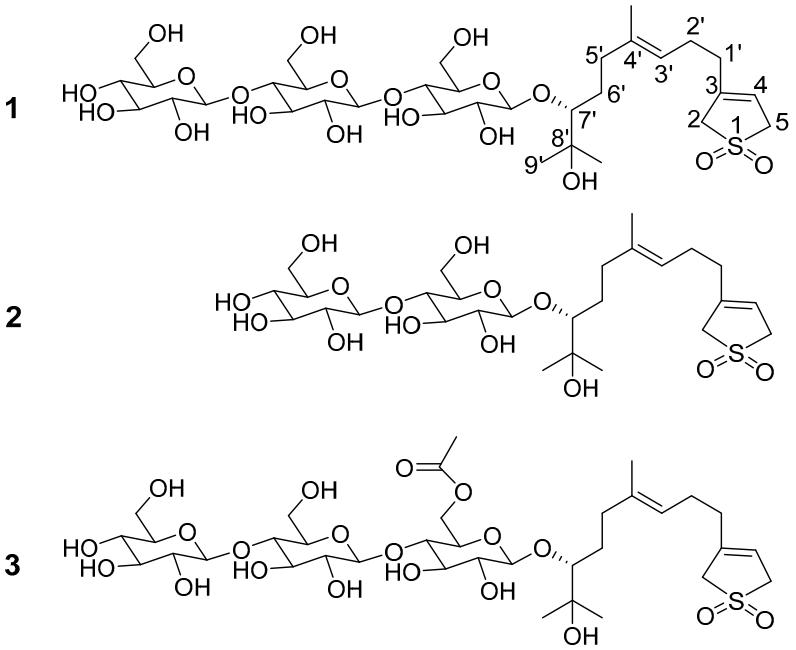

Figure 1. The chemical structures of compounds **1**–**3**.

Compound **1** was obtained as a white amorphous powder and the molecular formula $C_{33}H_{56}O_{19}S$ was indicated by HR-ESIMS at *m*/*z* 787.3065 $[M - H]^-$ (calcd. for $C_{33}H_{55}O_{19}S$ 787.3058). The ^{1}H-NMR (600 MHz) spectrum of **1** (Table 1) showed three tertiary methyl group signals (δ_H 1.37, 1.32, 1.61), two olefinic protons (δ_H 5.58, 5.34 (br t, *J* = 7.2 Hz)), as well as signals for three anomeric protons at (δ_H 4.89 (d, *J* = 7.9 Hz), 5.12 (d, *J* = 7.8 Hz), and 5.15 (d, *J* = 7.8 Hz)). The ^{13}C combined with HSQC NMR spectra of **1** indicated a structure with a total of 33 C-atom signals. Fifteen of them were attributed to the aglycone carbons including four olefinic carbons (δ_C 117.8, 138.7, 123.6, 136.8), one oxygenated methine carbon (δ_C 90.0), one oxygenated quaternary carbon (δ_C 71.9), two sulfonated methylene carbons (δ_C 57.4, 58.0), four sp3 methylene carbons (δ_C 25.6, 33.1, 36.2, 30.8), and three tertiary methyl carbons (δ_C 16.1, 25.3, 26.8), while the remaining carbon signals were characteristic to three glucosyl 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 ^{1}H−^{1}H 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 the NMR data of **1** with (2,3-*S*-*trans*,10*R*,6*E*)-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 [14] suggested that they shared the same sugar chain. The sugar moiety was further assigned by HSQC, HMBC and ^{1}H–^{1}H 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 that 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

of α-, β-(pro-*S* side), and β-(pro-*R* side) carbons of secondary alcohols to which glucosyl moieties were attached [16]. Furthermore, the ^{13}C chemical shifts at C-8 and C-12 of **1** were quite similar to those of (2,3-*S*-trans,10*R*,6*E*)-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 (10*R*) [14], 7,11-dimethyl-3-methylene-1,6-dodecadien-10,11-diol 10-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranoside (10*R*) [13], and icariside C_4 (10*R*) [17], while different from those of icariside C_1 [17]. The assignments at C-8 and C-9 of icariside C_4 and icariside C_1 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.

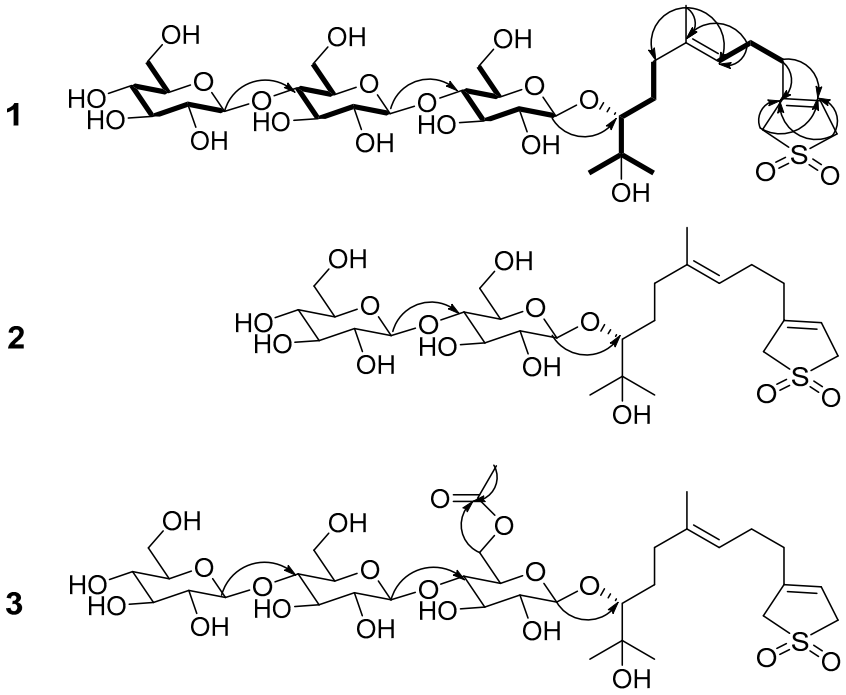

Figure 2. Key HMBC (arrows) and $^{1}H-^{1}H$ COSY (thick lines) correlations of compounds **1**–**3**.

Compound **2** was obtained as a white amorphous powder and its molecular formula $C_{27}H_{46}O_{14}S$ was established by HR-ESIMS at *m*/*z* 625.2515 $[M - H]^-$ (calcd. for $C_{27}H_{46}O_{14}S$ 625.2530). Comparing the NMR and MS data of **2** with **1**, it was determined that **2** had the same aglycone as **1**. The ^{13}C-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 4.91) 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 $C_{35}H_{58}O_{20}S$ was deduced by HR-ESIMS at *m*/*z* 829.3126 $[M - H]^-$ (calcd. for $C_{35}H_{58}O_{20}S$ 829.3164). The NMR data of **3** were very similar to **1**, except for the presence of the CH_3CO group. Furthermore, the CH_3CO 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-$CH_3\underline{C}O$ (δ 170.8). The sugar moieties of **3** were further assigned by the HSQC, HMBC, and $^{1}H-^{1}H$ 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. ^{1}H-NMR, ^{13}C-NMR data for compounds **1**–**3**.

No.	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	58.0	3.85 (2H, m)	58.0	3.85 (2H, m)	58.0	3.85 (2H, m)
3	138.7		138.7		138.7	
4	117.8	5.58 (1H, m)	117.8	5.58 (1H, m)	117.8	5.58 (1H, m)
5	57.4	3.89 (2H, m)	57.4	3.89 (2H, m)	57.4	3.89 (2H, m)
1′	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		136.8		136.8	
5′	36.2	2.74 (1H, m)	36.1	2.74 (1H, m)	36.1	2.74 (1H, m)
		2.50 (1H, m)		2.50 (1H, m)		2.50 (1H, m)
6′	30.8	1.84 (1H, m)	30.8	1.84 (1H, m)	30.8	1.84 (1H, m)
		1.76 (1H, m)		1.76 (1H, m)		1.76 (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)	90.4	3.75 (1H, dd, *J* = 1.5, 9.5Hz)
8′	71.9		71.9		71.9	
9′	25.3	1.37 (3H, s)	25.3	1.37 (3H, s)	25.5	1.37 (3H, s)
8′-CH_3	26.8	1.32 (3H, s)	26.8	1.32 (3H, s)	26.7	1.32 (3H, s)
4′-CH_3	16.1	1.61 (3H, s)	16.1	1.61 (3H, s)	16.1	1.61 (3H, s)
Glc-1′	105.7	4.89 (1H, d, *J* = 7.8 Hz)	105.7	4.91 (1H, d, *J* = 7.8Hz)	105.6	4.86 (1H, d, *J* = 7.8 Hz)
Glc-2′	74.3	4.05 (1H, o)	74.8	4.05 (1H, o)	71.9	4.23 (1H, o)
Glc-3′	76.5	4.22 (1H, o)	78.5	4.00 (1H, o)	76.5	4.22 (1H, o)
Glc-4′	80.9	4.28 (1H, o)	81.3	4.31 (1H, o)	81.3	4.23 (1H, o)
Glc-5′	76.4	3.95 (1H, o)	76.8	4.28 (1H, o)	76.8	4.00 (1H, o)
Glc-6′	61.9	4.47 (1H, o)	62.0	4.50 (2H, o)	62.1	4.58 (1H, o)
		4.47 (1H, o)				4.44 (1H, o)
Glc-1′′	105.0	5.12 (1H, d, *J* = 7.8 Hz)	105.0	5.18 (1H, d, *J* = 7.8Hz)	105.0	5.00 (1H, d, *J* = 7.8 Hz)
Glc-2′′	74.7	4.05 (1H, o)	75.0	4.05 (1H, o)	74.2	4.03 (1H, o)
Glc-3′′	76.7	4.22 (1H, o)	78.3	4.17 (1H, o)	78.5	4.01 (1H, o)
Glc-4′′	80.9	4.28 (1H, o)	71.6	4.17 (1H, o)	81.5	4.00 (1H, o)
Glc-5′′	78.5	3.96 (1H, o)	76.6	3.95 (1H, o)	73.2	4.13 (1H, o)
Glc-6′′	61.8	4.49 (1H, o)	62.5	4.52 (1H, dd, *J* = 2.4, 11.4 Hz)	64.3	5.13 (1H, dd, *J* = 2.4, 11.4 Hz)
		4.49 (1H, o)		4.29 (1H, o)		4.77 (1H, o)
Glc-1′′′	104.5	5.15 (1H, d, *J* = 7.8 Hz)			104.7	5.13 (1H, d, *J* = 7.8 Hz)
Glc-2′′′	75.0	4.05 (1H, o)			74.8	4.05 (1H, o)
Glc-3′′′	76.6	4.17 (1H, o)			76.6	3.95 (1H, o)
Glc-4′′′	71.5	4.17 (1H, o)			71.6	4.15 (1H, o)
Glc-5′′′	78.2	3.95 (1H, o)			78.2	4.17 (1H, o)
Glc-6′′′	62.5	4.52 (1H, dd, *J* = 2.4, 11.4 Hz)			62.5	4.52 (1H, dd, *J* = 2.4, 11.4 Hz)
		4.29 (1H, o)				4.28 (1H, o)
CH3CO					20.8	2.08 (3H, s)
CH3CO					170.8	

δ in C_5D_5N, in ppm from tetramethylsilane (TMS), ^{1}H-NMR at 600 MHz, ^{13}C-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 ^{1}H-NMR and 150 MHz for ^{13}C-NMR, PaloAlto, CA, USA) in pyridine-d_5 (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_2O (5:95, 30:70, 50:50, 75:25 and 95:10, *v*/*v*) to yield five factions (Fr. A−Fr. E). Fr. C (120 g) was subjected to silica-gel CC with a gradient mixture of $CHCl_3$:MeOH:H_2O (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_2O (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_2O (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_2O (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_2O (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_2O (20:80, *v*/*v*) to yield **3** (15 mg).

*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**): White amorphous power; $[\alpha]_D^{20}$ −10.5° (*c* 0.085, MeOH); IR (KBr) ν_{max} 3408, 2970, 2927, 1642, 1382, 1306, 1236, 1159, 1072, 1026; ^{1}H-NMR and ^{13}C-NMR spectroscopic data, see Table 1; HR-ESI-MS (positive) *m*/*z* 789.3241 $[M + H]^+$ (calcd. for $C_{33}H_{55}O_{19}S$ 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′-Dihydroxy-4′,8′-dimethyl-3′-nonenyl)-2,5-dihydro-1,1-dioxo-thiophen 7′-O-β-*D*-glucopyranosyl-(1→4)-O-β-*D*-glucopyranoside* (**2**): White amorphous power; $[\alpha]_D^{20}$ −10.3° (*c* 0.082, MeOH); IR (KBr) ν_{max} 3433, 2928, 1642, 1383, 1306, 1237, 1074, 1041; ^{1}H-NMR and ^{13}C-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′-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 power; $[\alpha]_D^{20}$ −12.5° (*c* 0.088, MeOH); IR (KBr) ν_{max} 3428, 2970, 2926, 1736, 1644, 1383, 1307, 1239, 1161, 1072, 1029; ^{1}H-NMR and ^{13}C-NMR spectroscopic data, see Table 1; HR-ESIMS (positive) *m*/*z* 831.3329 $[M + H]^+$ (calcd. for $C_{35}H_{59}O_{20}S$ 831.3320), 853.3165 $[M + Na]^+$, 669.2820 $[M + H - Glc]^+$, 507.2261 $[M + H - Glc - Glc]^+$, 303.1612 $[M + H - Glc - Glc - (Ac\text{-}Glc)]^+$.

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. CF_3COOH (5 mL) for 5 h at 95 °C, respectively. After extraction with CH_2Cl_2 (3 × 5 mL), the aq. layer was repeatedly evaporated to dryness with EtOH until neutral, and then analyzed by TLC over silica gel ($CHCl_3$:MeOH:H_2O, 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.

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