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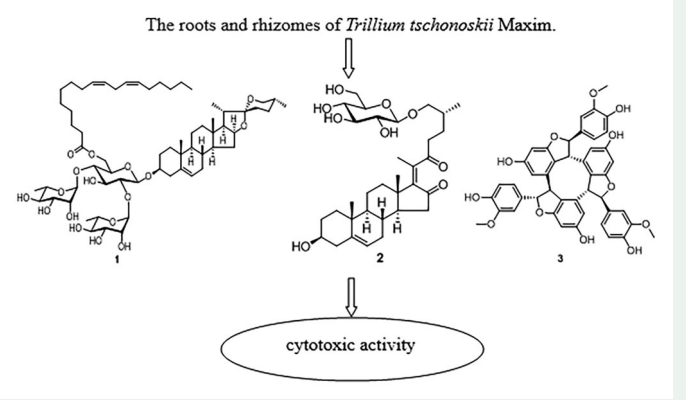
Chemical constituents of *Trillium tschonoskii* Maxim.

Ting Yan, Anhua Wang, Gaosheng Hu and Jingming Jia

School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, China

ABSTRACT

A new fatty acid-spirostan steroid glycoside ester, a new cholestane glycoside and a new stilbene trimer, along with three known steroidal saponins, were isolated from the 70% EtOH extract of the roots and rhizomes of *Trillium tschonoskii* Maxim. The structure of isolated compounds was elucidated by spectroscopic analysis. Compound **1–6** were assessed for their cytotoxicity against cancer cell lines (MCF-7, HCT-116, DU-145, SGC-7901, MCF-7/ADR, K562/ADR), and the result showed that compound **4** was highly toxic to six human tumor cell lines.



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1. Introduction

Trillium tschonoskii Maxim. (family Liliaceae) is a herbaceous plant in mid-western China, locally known as 'Yan Ling Cao', meaning that it is a herb that can prolong human life (Li et al. 2005). Its dried roots and rhizomes are used as a folk medicine for the treatment of neurasthenia, headache, cancer, and ameliorating pains, especially in the treatment of various inflammatory diseases (Fu 1992; Khan et al. 2018). Previous phytochemical and pharmacological investigations have shown that the main bioactive ingredients of *Trillium tschonoskii* were steroidal saponins (Chai et al. 2014; Wei et al. 2012; Khan et al. 2019). A number of saponins from the plant could inhibit the growth or/and the metastasis of tumors (Li et al. 2014; Wang et al. 2013).

CONTACT Jingming Jia jiajingming@163.com

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During our recent chemical investigation on this plant, a new fatty acid-spirostan steroid glycoside ester, a new cholestane glycoside and a new stilbene trimer, along with three known steroidal saponins, were isolated from the roots and rhizomes of *Trillium tschonoskii* Maxim. (Figure S1). Additionally, the cytotoxicity of six compounds were evaluated against selected cancer cell lines (MCF-7, HCT-116, DU-145, SGC-7901, MCF-7/ADR, K562/ADR). Details of the isolation, structural elucidation, as well as the cytotoxicity of these compounds are presented below.

2. Results and discussion

2.1. Structure elucidation

Compound **1** showed a protonated ion $[M+H]^+$ at m/z 1131.7152(calc. for 1131.7190) in its HR-ESI-MS (Seeing Figure S7), which indicated the neutral formula of $C_{63}H_{102}O_{17}$. The resonances in the ^{13}C-NMR spectrum (Seeing Figure S2) of **1** were ascribed to a steroid skeleton, three sugar moieties, and a saturated fatty acid unit. The IR spectrum indicated hydroxyl and ester carbonyl functions due to absorptions at 3386 and 1739 cm^{-1}, respectively. The ^{13}C-NMR spectra exhibited an ester carbonyl carbon signal δ_C 173.3 (s, C-1‴), three groups of double bond carbon signals δ_C 121.8 (d, C-6), 128.5 (2 × d), 130.3 (d), 130.5 (d) and 140.9 (s, C-5), three sugar anomeric carbon signals δ_C 100.6 (d, C-1′), 102.2 (d, C-1″) and 103.2 (d, C-1‴), seven methyl carbon signals δ_C 14.3 (q, C-18⁗), 15.1 (q, C-21), 16.4 (q, C-18), 17.4 (q, C-27), 18.5 (q, C-6‴), 18.7 (q, C-6″) and 19.4 (q, C-19) and a group of long chain methylene carbon signals δ_C 29.4-30.1 (t). The ^{1}H-NMR spectrum (Seeing Figure S1) showed seven methyl hydrogen signals, δ_H 1.62 (3 H, d, $J = 6.2$ Hz, H-6‴) and 1.75 (3 H, d, $J = 6.1$ Hz), H-6″) were two characteristic rhamnose methyl signal, δ_H 0.85 (3 H, t, $J = 6.5$ Hz, H-18⁗) was a long-chain fatty acid terminal methyl signal, two secondary methyls δ_H 0.68 (3 H, d, $J = 5.6$ Hz, H-27), 1.14 (3 H, d, $J = 6.8$ Hz, H-21), two tertiary methyls δ_H 0.83 (3 H, s, H-18), 1.03 (3 H, s, H-19) and the characteristic proton signal δ_H 2.92 (2 H, t, $J = 5.8$ Hz, H-11″) on the carbon attached to the two double bonds in the linoleic acid structural fragment. By ^{1}H, ^{13}C-NMR and HSQC spectra (Seeing Figure S4), the following signals were also observed: one oxidized quaternary carbon signal δ_C 109.3 (C-22), two oxidized methine signals δ_H/δ_C 3.86/78.8 (CH-3) and 4.55/81.1 (CH-16). Based on the characteristics of the above spectral data and the degree of unsaturation, compound **1** should be a steroidal saponin compound with linoleic acid ester which has a C_{27} spirooxane, three sugar fragments, and one linoleic acid structural unit. The location of glucose at C-3, two rhamnose at C-2′ and C-4′ positions of glucose was determined by HMBC correlations between H-1′(δ_H4.91)/C-3(δ_C78.8), H-1″(δ_H6.35)/C-2′(δ_C77.6) and H-1‴(δ_H5.52)′/C-4′ (δ_C 79.6), respectively.

According to relevant literature research, the characteristics of the above nuclear magnetic signals have higher similarity with the data of progenin III linoleate (Zulfiqar, et al. 2013) and compound **6** dioscin (Ju and Jia 1992) reported in recent years. Compared with dioscin, only a group of linoleic acid signals were observed in compound **1**, which was highly consistent with the linoleic acid signal in progenin III linoleate. It suggested that the linoleic acid fragment was also attached to the C-6′ of glucose. By comparing the carbon spectrum data of compound **1**

and dioscin, it was found that C-6′ in compound **1** shifted to low field and C-5′ shifted to high field. In the HMBC spectrum of **1** (Seeing Figure S3), a long-range correlation between H-6′a (δ_H 4.57), H-6′b (δ_H 4.71) and C-1′′′′(δ_C 173.3), which confirmed that the linoleic acid fragment was linked to the C-6′ of glucose. According to HSQC, HMBC (Seeing Figure S8A), ROESY (Seeing Figure S8B), the stereochemistry of compound **1** was found to be similar to that of progenin III linoleate and dioscin by nuclear magnetic signal characteristics (hydrocarbon spectrum chemical shift, hydrogen signal coupling constant). Therefore, the structure of compound **1** was determined as: dioscin 6′-linoleate.

Compound **2** was obtained as a light yellow amorphous powder. The IR spectrum (Seeing Figure S14) revealed the presence of OH (3423 cm^{-1}), C=O bonds (1717 cm^{-1}), and C=C bonds (1631 cm^{-1}). The molecular formula was established as $C_{33}H_{50}O_9$ by the HR-ESI-MS data m/z 613.3348 $[M+Na]^+$ (calc. for 613.3347) (Seeing Figure S15). The ^{1}H- and ^{13}C-NMR spectra (Seeing Figure S8,S9) exhibited four characteristic Me signals δ_H 1.13(3 H, s, H-18), 0.94 (3 H, d, $J = 6.4$ Hz, H-27), 1.08(3 H, s, H-19) and 1.95 (3 H, s, H-21); and δ_C 15.9(C-21), 17.1(C-18), 17.4(C-27), 19.8(C-19); Glucose anomeric H-atom signals at δ_H 4.23 (1 H, d, $J = 7.8$ Hz). The ^{13}C-NMR data of **2** showed 33 C-atom signals including one monosaccharide unit with 6 carbons, so the molecular formula of the aglycon was $C_{27}H_{41}O_3$, indicating that the aglycon should be a steroid skeleton containing three oxygen atoms.

According to HMBC (Seeing Figure S16A), ROESY (Seeing Figure S16B), the long-distance correlations Me(19) (δ_H1.08)/C(5) (δ_C 142.5); H-C(6) (δ_H 5.36)/C(4) (δ 43.0) and C(7) (δ_C 32.5); and Me(18) (δ_H 1.13) and Me(21) (δ_H 1.95) with C(17) (δ_C 143.9) and C(20)(δ_C 146.9) in the HMBC experiment (Seeing Figure S11) of **2** demonstrated that a C=C bond should be placed at C(5)/C(6) and another C=C bond at C(17)/C(20) in **2**.

In addition, the ^{13}C-NMR spectrum of **2** showed two CO signals which were assigned to C(16) (δ_C 207.9) and C(22) (δ_C 213.9) by the correlations in the HMBC spectrum H-C(15) (δ_H 2.08, 2.17) and Me(21) (δ_H 1.95) with C(16) (δ_C 207.9), and H-C(24) (δ_H1.50,1.78) and Me(21) (δ_H 1.95) with C(22) (δ_C 213.9), respectively.

The (R)-configuration at C(25) in **2** was deduced by the H-atom resonances of CH_2(26) (δ_H 3.73 for H_b-C(26) and δ_H 3.39 for H_a-C(26)), which showed a (δ_{H26b}–δ_{H26a})= 0.34 ppm which is $<$ 0.48 (Agrawal 2005). By comparing the NMR data of the aglycone of **2** with those of Parispseudoside C(Xiao et al. 2009) the aglycone was identified as (*25 R*)-3,26-dihydroxycholesta-5,17(20)-diene-16,22-dione.

The correlation between H-C(1) (δ_H 4.23) of glucopyranosyl unit with C(26) (δ_C 75.8) of the aglycone in the HMBC experiment indicated that the glucopyranosyl unit was linked to C(26) of the aglycone of 2.

Acid hydrolysis of 2 with 2 M HCl obtained D-glucose as carbohydrate moiety, which was characterized by comparison of the retention times and the positive peak in the HPLC analysis with the corresponding authentic samples (Seeing Figure S25). By the HPLC analysis with a JAsco OR-4090 detector, D-glucose has the same retention time as L-glucose, but D-glucose is a positive peak and L-glucose is a reversed peak. The glucopyranoside was configuration on the ground of the coupling constants ($^3J_{1,2} > 7.0$ Hz) of the anomeric protons. Therefore, the structure of 2 was elucidated as(25*R*)-3,26-dihydroxycholesta-5,17(20)-diene-16,22-dione-26-*O*-*D*-glucopyranoside, and named Trilliumoside.

Compound **3** was obtained as a light yellow amorphous powder with molecular formula of $C_{45}H_{36}O_{12}$ determined by its HRESI-MS at *m/z* 767.2149 $[M\text{-}H]^-$ (calc. for 767.2129) (Seeing Figure S22). The IR spectrum revealed the presence of OH (3396 cm^{-1}) and benzene ring (1616 cm^{-1}, 1518 cm^{-1}) (Seeing Figure S23). The ^{1}H-NMR spectrum (Seeing Figure S17) of **3** showed signals for three methoxy at δ_H 3.63 (3 H × 2, s), 3.75 (3 H, s); three groups of 1,2,4-trisubstituted benzene rings at δ_H 6.65 (1 H, d, J = 1.7 Hz, H-2a)/6.62 (1 H, dd, J = 8.1, 1.7 Hz, H-6a)/6.70 (1 H, d, J = 8.1 Hz, H-5a), δ_H 6.78 (1 H, d, J = 1.7 Hz, H-2b)/6.76 (1 H, dd, J = 8.1, 1.7 Hz, H-6b)/6.70 (1 H, d, J = 8.1 Hz, H-5b) and δ_H 6.48 (1 H, d, J = 1.8 Hz, H-2c)/6.56 (1 H, dd, J = 8.1,1.8 Hz, H-6c)/6.67(1H, d, J = 8.1 Hz, H-5c); three groups of 1,2,3,5-tetrasubstituted benzene rings at δ_H 6.22 (1 H, d, J = 1.8 Hz, H-12a)/6.67 (1 H, d, J = 1.8 Hz, H-14a), δ_H 6.15 (1 H, d, J = 1.9 Hz, H-12b)/6.46 (1 H, d, J = 1.9 Hz, H-14b) and δ_H 6.23 (1 H, d, J = 2.0 Hz, H-12c)/5.87 (1 H, d, J = 2.0 Hz, H-14c).

A total of 45 carbon signals were detected in its ^{13}C-NMR spectrum (Seeing Figure S18). Combined with its HSQC spectrum analysis (Seeing Figure S20), it was confirmed that in addition to the 36 aromatic carbon signals from the six benzene rings, there were also three methoxy carbon signals and the 6 methine carbon signals. According to the characteristics of the above spectral data and the degree of unsaturation, compound **3** should be a stilbene trimer compound which has four rings in addition to six benzene rings. According to the literature (Tetsuro et al. 2012), the nuclear magnetic resonance signal characteristics of compound **3** were very similar to those of cyperusphenol D, except that **3** contains three extra methoxy signals. According to HMBC (Seeing Figure S24), the long-distance correlations δ_H 3.63 (3 H, s)/δ_C149.0 (s, C-3c); δ_H 3.63 (3 H, s)/δ_C149.1(s, C-3b); δ_H 3.75 (3 H, s)/δ_C148.8 (s, C-3a) in the HMBC spectrum indicated that the three extra methoxy groups were attached to the C-3a, C-3b, and C-3c positions, respectively. In addition, the connection method is further verified by H-2a/δ_H 3.75 (3 H, s), H-2b/δ_H 3.63 (3 H × 2, s), H-2c/δ_H 3.63 (3 H × 2, s) in ROESY (Seeing Figure S21 and Figure S24).

Comparison of ^{1}H- and ^{13}C-NMR spectral data and the hydrogen signal coupling constant with cyperusphenol D indicated that compound **3** and cyperusphenol D had a consistent relative configuration. Therefore, the structure of Compound **3** was named 3a, 3b, 3c-tri-O-methylcyperusphenol D.

According to the previous reports, the other compounds were identified to be Chonglouosid(**4**) (Masateru et al. 2007, Liu et al. 2008), pennogenin-3-*O*-α-*L*-rhamnopyranosyl (1→4)-[α-*L*-rhamnopyranosyl(1→2)]-β-*D*-glucopyranoside (**5**) (Nakano et al. 1989), dioscin (**6**) (Ju and Jia 1992) on the basis of spectroscopic data and past literature.

The inhibitory effect of isolated compounds against tumor cell lines (MCF-7, HCT-116, DU-145, SGC-7901, MCF-7/ADR,K562/ADR) were investigated using an *in vitro* bioassay. The result showed that the IC_{50} values of the positive control 5-fluorouracil were 12.16, 2.58, 2.92, 14.5, >100, and >100 μM, respectively. Compared with the positive control 5-fluorouracil, compound **4** was highly toxic to six human tumor cell lines with the IC_{50} values 3.15, 0.81, 1.67, 2.68, 2.34 and 2.92 μM, respectively.

3. Experimental

3.1. General experiment procedure

Optical rotations were determined using a MCP-200 (Anton Paar, Germany). UV spectra were recorded on a Shimadzu-2201 (Kyoto, Japan). The IR spectrum was obtained

from a Bruker IFS-55 spectrophotometer (Karlsruhe, Germany) using KBr pellet. HR-ESI-MS data were performed on a Xevo G2 QTof (Waters MS Technologies, Manchester, UK). NMR spectra were run on a Bruker AV-400 spectrometer (Karlsruhe, Germany). Semi-preparative HPLC was performed on a YMC-Pack ODS-A column(10 × 250 mm, 5 μm; YMC-Pack, Japan), equipped with a LC-3000A pump (Beijing Chuang Xin Tong Heng Science and Technology Co., Ltd., China) and a 3000 UV–vis detector (Beijing Chuang Xin Tong Heng Science and Technology Co., Ltd., China). Sugars analytical HPLC was carried out on a Jasco PU-4180 pump (Kyoto, Japan) and a OR-4090 detector (Kyoto, Japan). HPLC was performed with an Asahipak NH2P-50 4E column (4.6 mm × 250 mm, 5 μm, Shodex, Japan).

3.2. Plant material

The dried roots and rhizomes were collected in July 2014 from Shennongjia, Hubei Province, China, and were identified by associate professor Jia Lingyun (School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, China). The voucher sample (JTZ-20140712) has been deposited in the Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang, China.

3.3. Extraction an isolation

The dried roots and rhizomes (1.7 kg) of *Trillium tschonoskii* Maxim. were powered and extracted three times with 70% EtOH (each 2 h) and the combined solution evaporated to dryness by a vacuum rotary evaporator to afford a syrup (600 g).The crude extract was suspended with H_2O (1.0 L) and successively partitioned with petroleum ether, ethyl acetate and *n*-butanol to yield three layers of extracts. The *n*-butanol extract (210 g) was fractionated by silica gel column chromatography eluting with CH_2Cl_2-MeOH (100:0–0:100) to obtain thirteen fractions (Fr. a-m) based on TLC analyses. Fr. f was subjected to ODS CC eluted with MeOH-H_2O (10:90-100:0) to give ten subfractions f1–f10. Subfraction f6 was dealed with semi-preparative RP-HPLC to yield **2** (2.3 mg). Fr. h was dealed with preparative HPLC to yield compounds **5** (156.3 mg) and **6** (392.0 mg). Fr. i was purified by preparative HPLC to afford compounds **4** (43.3 mg).

The ethyl acetate extract (90 g) was dealed with a silica gel column by elution with CH_2Cl_2–MeOH (100:0-1:1) in sequence to give fractions A–G. Fr. F was separated in a silica gel column by elution with CH_2Cl_2–MeOH (8:1-1:1) in sequence to give fractions F1-F8. F1 was purified by Sephadex LH-20 in sequence to give fractions F1a–F1h. F1d was purified by recrystallization to yield compounds **1** (12.6 mg) . Fr. C was separated in a silica gel column by elution with P.E–Acetone (8:1-1:1) in sequence to give fractions C1-C7. Fr.C7 was dealed with a silica gel column by elution with CH_2Cl_2–MeOH (50:1-10:1) in sequence to give fractions C7a–C7e. C7b was purified by preparative TLC to yield compounds **3** (13.3 mg). dioscin 6′-linoleate: light yellow amorphous powder; $[\alpha]^{20}_{D}$–98 (*c* 0.10, C_5H_5N); HR-ESI-MS *m/z* 1131.7152 $[M+H]^+$ (calc. for $C_{63}H_{103}O_{17}$, 1131.7190); IR (KBr) ν_{max} 3747, 3386, 2926, 2854, 2111, 1739, 1631, 1541, 1522, 1457, 1379, 1243, 1134, 1055, 982, 919, 901, 839, 812, 721 and 635 cm^{-1}; ^{1}H-NMR (400 MHz, pyridine-d_5) δ_H 1.03 (1 H, m, H-1a),

1.76 (1 H, m, H-1b), 1.86 (1 H, m, H-2a), 2.13 (1 H, m, H-2b), 3.86 (1 H, m, H-3), 2.70 (1 H, m, H-4a), 2.79 (1 H, m, H-4b), 5.32 (1 H, br s, H-6),1.50 (1 H, m, H-7a), 1.89 (1 H, m, H-7b), 1.68 (1 H, m, H-8), 0.92 (1 H, m, H-9),1.42 (1 H, m, H-11a), 1.49 (1 H, m, H-11b), 1.11 (1 H, m, H-12a), 1.71 (1 H, m, H-12b), 1.07 (1 H, m, H-14), 1.50 (1 H, m, H-15a), 2.04 (1 H, m, H-15b), 4.55 (1 H, m, H-16), 1.81 (1 H, dd, $J = 8.5$, 6.5 Hz, H-17), 0.83 (3 H, s, H-18), 1.03 (3 H, s, H-19), 1.95 (1 H, m, H-20), 1.14 (3 H, d, $J = 6.8$ Hz, H-21), 1.56–1.78 (2 H, m, H-23), 1.29 (1 H, m, H-24a), 1.57(1 H, m, H-24b), 1.57 (1 H, m, H-25), 3.49 (1 H, t, $J = 10.4$ Hz, H-26a), 3.58 (1 H, br d, $J = 10.4$ Hz, H-26b), 0.68 (3 H, d, $J = 5.6$ Hz, H-27), 4.91 (1 H, d, $J = 7.6$ Hz, H-1′), 4.22 (1 H, dd, $J = 9.0$, 7.6 Hz, H-2′), 4.18 (1 H, t, $J = 9.0$ Hz, H-3′), 4.09 (1 H, t, $J = 9.2$ Hz, H-4′), 3.82 (1 H, m, H-5′), 4.57 (1 H, m, H-6′a), 4.71 (1 H, br d, $J = 11.8$ Hz, H-6′b), 6.35 (1 H, s, H-1″), 4.80 (1 H, br s, H-2″), 4.61 (1 H, dd, $J = 9.4$, 3.3 Hz, H-3″), 4.35 (1 H, t, $J = 9.4$ Hz, H-4″), 4.92 (1 H, m, H-5″), 1.75 (3 H, d, $J = 6.1$ Hz, H-6″), 5.52 (1 H, s, H-1‴), 4.63 (1 H, br s, H-2‴), 4.49 (1 H, dd, $J = 9.3$, 3.0 Hz, H-3‴), 4.32 (1 H, t, $J = 9.3$ Hz, H-4‴), 4.82 (1 H, m, H-5‴), 1.62 (3 H, d, $J = 6.2$ Hz, H-6‴), 2.34 (2 H, t, $J = 7.5$ Hz, H-2⁗), 1.62 (2 H, m, H-3⁗), 2.10 (2 H, m, H-8⁗/H-14⁗), 5.46-5.51 (4 H, m, H-9⁗/H-10⁗/H-12⁗/H-13⁗), 2.92 (2 H, t, $J = 5.8$ Hz, H-11⁗), 1.26 (2 H, m, H-16′⁗), 1.25 (2 H, m, H-17′⁗), 0.85 (2 H, t, $J = 6.5$ Hz, H-18′⁗), 1.18-1.37(2H, m, other CH_2). ^{13}C-NMR (100 MHz, pyridine-d_5) δ_C 37.6 (t, C-1), 30.2 (t, C-2), 78.8 (d, C-3), 39.1 (t,C-4), 140.9 (s,C-5), 121.8 (d,C-6), 32.4 (t,C-7), 31.9 (d,C-8), 50.4 (d,C-9), 37.2 (s,C-10), 21.2 (t,C-11), 39.9 (t,C-12), 40.5 (s,C-13), 56.7 (d,C-14), 32.3 (t,C-15), 81.1 (d,C-16), 63.0 (d,C-17),16.4 (q,C-18),19.4 (q,C-19),42.0 (d,C-20),15.1 (q,C-21), 109.3 (s,C-22), 31.7(t,C-23), 29.3(t,C-24), 30.6 (d,C-25), 66.9 (t,C-26), 17.4 (q,C-27), 100.6 (d,C-1′), 77.6 (d,C-2 ′), 77.7 (d,C-3′),79.6 (d,C-4′), 73.6 (d,C-5′), 63.3 (t,C-6), 102.2 (d,C-1″), 72.5 (d,C-2″), 72.8 (d,C-3″), 74.1 (d,C-4″), 69.6 (d,C-5″), 18.7 (q,C-6″), 103.2 (d,C-1‴), 72.4 (d,C-2‴), 72.7 (d,C-3‴), 73.7 (d,C-4‴), 70.8 (d,C-5‴), 18.5 (q,C-6‴), 173.3 (s,C-1⁗), 34.4 (t,C-2⁗), 25.3 (t,C-3⁗),27.6 (t, C-8⁗/C-14⁗), 128.5,128.5,130.3,130.5 (d, C-9⁗, C-10⁗, C-12⁗,C-13⁗), 26.1 (t, C-11⁗), 32.2 (t,C-16′⁗), 23.0 (t,C-17′⁗), 14.3 (q,C-18′⁗), 29.4–30.1 (t, other CH_2).

Trilliumoside: light yellow amorphous powder; $[\alpha]^{20}_D$ – 79.0 (*c* 0.30, MeOH); HR-ESI-MS data m/z 613.3348 $[M + Na]^+$ (calc. for $C_{33}H_{50}O_9$ 613.3347); UV (MeOH) λ_{max} 250 nm; IR (KBr) ν_{max} 3423, 2925, 2853, 1717, 1631, 1463, 1383, 1264, 1169, 1043, 616 cm^{-1}. ^{1}H-NMR (600 MHz, MeOH) δ_H 1.10 (1 H, m, H-1a), 1.89 (1 H, m, H-1b), 1.80 (1 H, m, H-2a), 1.99 (1 H, m, H-2b), 3.40 (1 H, m, H-3), 2.22 (1 H, m, H-4a), 2.25 (1 H, m, H-4b), 5.36 (1 H, br d, $J = 4.5$ Hz, H-6), 1.65 (1 H, m, H-7a), 1.99 (1 H, m, H-7b), 1.80 (1 H, m, H-8), 1.15 (1 H, m, H-9), 1.70-1.87 (2 H, m, H-11), 1.75 (1 H, m, H-12a), 2.35 (1 H, m, H-12b), 1.60 (1 H, m, H-14), 2.08 (1 H, m, H-15a), 2.17 (1 H, m, H-15b), 1.13(3 H, s, CH_3-18), 1.08(3 H, s, CH_3-19), 1.95 (3 H, s, CH_3-21), 2.58 (2 H, t, $J = 7.4$ Hz, H-23), 1.50 (1 H, m, H-24a), 1.78(1 H, m, H-24b), 1.80 (1 H, m, H-25), 3.39 (1 H, dd, $J = 9.5$, 6.1 Hz, H-26a), 3.73 (1 H, dd, $J = 9.5$, 6.1 Hz, H-26b), 0.94 (3 H, d, $J = 6.4$ Hz, CH_3-27), 4.23 (1 H, d, $J = 7.8$ Hz, H-1′), 3.17 (1 H, dd, $J = 9.0$, 7.8, H-2′), 3.32 (1 H, m, H-3′), 3.26 (1 H, m, H-4′), 3.25 (1 H, m, H-5′), 3.65 (1 H, $J = 11.5$, 5.2 Hz, H-6′a), 3.85 (1 H, dd, $J = 11.5$,1.6 Hz, H-6′b). ^{13}C-NMR (150 MHz, MeOH) δ_C 38.2 (t, C-1), 32.2 (t,C-2), 72.3 (d,C-3), 43.0 (t,C-4), 142.5 (s,C-5), 121.9 (d,C-6), 32.5 (t,C-7), 32.0 (d,C-8), 51.2 (d,C-9), 37.8 (s,C-10), 21.9 (t,C-11), 37.3 (t,C-12), 44.6 (s,C-13), 52.1 (d,C-14), 38.6 (t,C-15), 207.9 (s,C-16), 143.9 (s,C-18), 17.1 (q,C-18), 19.8 (q,C-19), 146.9 (s,C-20), 15.9 (q,C-21), 213.9 (s,C-22), 39.3 (t,C-23), 28.3 (t,C-24), 34.0 (d,C-25), 75.8 (t,C-26), 17.4 (q,C-27), 104.6 (d,C-1′), 75.2 (d,C-2′), 78.2 (d,C-3′), 71.7 (d,C-4′), 77.9 (d,C-5′), 62.8 (t,C-6′).

3a,3b,3c-tri-O-methylcyperusphenol D: light yellow amorphous powder; $[\alpha]^{20}_{D}$ – 85.3 (*c* 0.60, MeOH); UV (MeOH) λ_{max}254 nm; IR (KBr) ν_{max}3396, 2922, 2851, 1616, 1518, 1490, 1452, 1434, 1365, 1273, 1240, 1211, 1161, 1126, 1114, 1074, 1033, 998, 836, 777, 685 and 634 cm^{-1}; HR-ESI-MS *m/z* 767.2149$[M-H]^-$ (calc. for calc. for $C_{45}H_{35}O_{12}$,767.2129). ^{1}H-NMR (400 MHz, CD_3OD) δ_H 6.65 (1 H, d, *J* = 1.7 Hz, H-2a), 6.70 (1 H, d, *J* = 8.1 Hz, H-5a), 6.62 (1 H, dd, *J* = 8.1, 1.7 Hz, H-6a), 6.10 (1 H, br s, H-7a), 3.97 (1 H, br s, H-8a), 6.22 (1 H, d, *J* = 1.8 Hz, H-12a), 6.67 (1 H, d, *J* = 1.8 Hz, H-14a), 6.78 (1 H, d, *J* = 1.7 Hz, H-2b), 6.70 (1 H, d, *J* = 8.1 Hz, H-5b), 6.76 (1 H, dd, *J* = 8.1, 1.7 Hz, H-6b), 5.86 (1 H, d, *J* = 9.4 Hz, H-7b), 4.69 (1 H, d, *J* = 9.4 Hz, H-8b), 6.15 (1 H, d, *J* = 1.9 Hz, H-12b), 6.46 (1 H, d, *J* = 1.9 Hz, H-14b), 6.48 (1 H, d, *J* = 1.8 Hz, H-2c), 6.67 (1 H, d, *J* = 8.1 Hz, H-5c), 6.56 (1 H, dd, *J* = 8.1, 1.8 Hz, H-6c), 4.69 (1 H, d, *J* = 7.1 Hz, H-7c), 4.50(1 H, d, *J* = 7.1 Hz, H-8c), 6.23 (1 H, d, *J* = 2.0 Hz, H-12c), 5.87 (1 H, d, *J* = 2.0 Hz, H-14c), 3.75 (3 H, s, 3a-OCH_3), 3.63 (3 H × 2, s, 3 b-OCH_3 and 3c-OCH_3). ^{13}C-NMR (100 MHz, CD_3OD) δ_C 133.3 (s,C-1a), 110.2 (d, C-2a), 148.8 (s, C-3a), 147.1 (s, C-4a), 116.0 (d, C-5a), 119.5 (d, C-6a), 87.1 (d, C-7a), 48.0 (d, C-8a), 140.0 (s, C-9a), 121.5 (s, C-10a), 161.0 (s, C-11a), 96.9 (d, C-12a), 159.7 (s, C-13a), 106.4 (d, C-14a), 133.7 (s,C-1b), 110.1 (d, C-2b), 149.1 (s C-3b), 147.7 (s, C-4b), 116.0 (d, C-5b), 120.3 (d, C-6b), 90.9 (d, C-7b), 53.2 (d, C-8b), 139.4 (s, C-9b), 120.2 (s, C-10b), 161.8 (s, C-11b), 97.1 (d, C-12b), 161.2 (s, C-13b), 106.0 (d, C-14b), 132.8 (s, C-1c), 110.8 (d, C-2c), 149.0 (s, C-3c), 147.9 (s, C-4c), 115.8 (d, C-5c), 120.6 (d, C-6c), 96.5 (d, C-7c), 56.6 (d, C-8c), 141.3 (s, C-9c), 119.0 (s, C-10c), 162.3 (s, C-11c), 98.4 (d, C-12c), 159.6 (s, C-13c), 109.1 (d, C-14c), 56.4 (3a-OCH_3), 56.3 (3 b-OCH_3 and 3c-OCH_3).

3.4. Hydrolysis of new compounds 1 and 2

Two milligram powder of compounds 1 and 2 were hydrolysed with 2 M HCl (2 mL) at 90 °C water bath for 5 h. Then, the reaction mixture was extracted with $CHCl_3$ (2 mL × 3). The water-layer was evaporated in vacuo by a rotatory evaporator, the residue was dissolved by H_2O, Subsequently, the H_2O fraction including saccharide was analyzed by a JAsco OR-4090 detector with comparing to standard substance glucose and rhamnose (chromatography column: Shodex Asahipak NH_2P-504E, CH_3CN:H_2O (3:1), 1.0 mL/min).

3.5. Cytotoxicity assay

Compounds 1–6 were tested for cytotoxicity against MCF-7, HCT-116, DU-145, SGC-7901, MCF-7/ADR and K562/ADR cancer cell lines by the standard microculture tetrazolium (MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay using 5-FU(5-Fluorouracil)as a positive control(Wang et al. 2014). The absorbance was surveyed at 570 and 630 nm on a microplate reader (SpectraMax M5, Molecular Devices, USA). The percentage of cell viability was computed relative to the absorbance intensity of control cells.

4. Conclusions

The present article has obtained 6 compounds from the roots and rhizomes of *Trillium tschonoskii* Maxim., including three new compounds. On the basis of the structure

analysis, the antitumor effects of these isolated compounds have been evaluated *in vitro* using MTT assay. Compared with the positive control 5-fluorouracil, the result showed that compound **4** was highly toxic to six human tumor cell lines.

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Disclosure statement

The authors declare that there are no conflicting interest.

ORCID

Anhua Wang http://orcid.org/0000-0002-8615-3424
Jingming Jia http://orcid.org/0000-0002-0779-4013

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