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Cytotoxic steroidal saponins from *Trillium kamtschaticum*

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ABSTRACT

Eight new steroidal saponins, trillikamtosides K–R (**1**–**8**), along with three known analogues, were isolated from the whole plants of *Trillium kamtschaticum*. Their structures were unambiguously established by interpretation of spectroscopic data (MS and NMR) and chemical methods. Compound **1** had a rare aglycone featuring a skeleton of 16-oxaandrost-5-en-3-ol-17-one, which was reported for the first time. The isolated saponins were tested for cytotoxicities against HCT116 cells, and trillikamtoside R (**8**) was found to show the most cytotoxic effect with an IC_{50} value of 4.92 μM.

Trillium kamtschaticum, a perennial herbaceous flowering plant of family Liliaceae, is widely distributed in Northeast Asia and grows under-forest of 500–1400 m altitude in Jilin Province of China.[1] Its rhizomes have been used as a traditional Chinese medicine for the treatment of hypertension, waist-leg pain, and traumatic haemorrhage.[2] Previous phytochemical and pharmacological investigations indicated that *T. kamtschaticum* is an abundant resource of steroidal saponins[3–6] with few information regarding their biological activity. Our recent study revealed that 70% EtOH elution from a macroporous resin of the crude extract of *T. kamtschaticum*[7] showed significant hemostatic effect and clarified that the bioactive constituents were pennogenin saponins (such as Tb, Tc, and Tg).[8] However, further HPLC analysis of the 30% EtOH elution disclosed that this fraction featured different chemical components from those in 70% EtOH elution. This stimulated us to conduct the phytochemicals of the 30% EtOH eluent by several chromatographic techniques.[9] As a result, eight new steroidal saponins, named trillikamtosides K–R (**1**–**8**), along with three known analogues (Fig. 1) were obtained and characterized. Structures of the known compounds were identified as Tf (**9**, 26-*O*-*β*-D-glucopyranosyl-17(20)-dehydrokryptogenin-3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*β*-D-glucopyranoside)[3], aethioside A (**10**),[10] and trikamsteroside E (**11**)[6] by comparison of their NMR and MS data with those reported values in the literature.

Trillikamtoside K (**1**)[11] was isolated as a white amorphous powder. Its molecular formula was assigned as $C_{30}H_{46}O_{12}$ by its HRESIMS peak at *m*/*z* 621.2890 $[M+Na]^+$ (calcd. for $C_{30}H_{46}O_{12}Na$, 621.2881) and ^{13}C NMR data, suggesting eight degrees of unsaturation. The 1D (^1H and ^{13}C) NMR and HSQC spectra (Table 1) of the aglycone moiety for **1** showed the presence of two methyls [δ_H 0.95 (3H, s), δ_C 13.9 (q, Me-18); δ_H 0.98 (3H, s), δ_C 19.3 (q, Me-19)], an oxygenated methylene [δ_H 4.18 (1H, dd, J = 8.4, 6.8 Hz) and 3.95 (1H, dd, J = 11.6, 8.4 Hz), δ_C 69.1 (t, C-15)], an oxygenated methine [δ_H 3.90 (1H, m), δ_C 77.8 (d, C-3)], a trisubstituted double bond [δ_H 5.22 (1H, br d, J = 4.8 Hz, H-6), δ_C 141.3 (s, C-5) and 120.7 (d, C-6)], and an ester carbonyl group [δ_C 181.2 (s, C-17)]. Besides, the presence of two sugar units was inferred by two anomeric protons at δ_H 5.02 (1H, d, J = 7.2 Hz, H-1′) and 6.36 (1H, br s, H-1″), which exhibited HSQC correlations with the relevant anomeric carbon signals at δ_C 100.4 (d, C-1′) and 102.1 (d, C-1″). The aforementioned double bond, carbonyl group, and the two monosaccharide units in **1** accounted for four degrees of unsaturation, and the remaining four ones required that the aglycone of **1** be a tetracyclic-ring system. Further analysis of HSQC, ^1H–^1H COSY, and HMBC experiments of **1** led to the construction of its aglycone gross structure (Fig. 2). Three substructural fragments (C-1–C-4, and C-6–C-9–C-11–C-12, and C-8–C-14–C-15) were established based on the ^1H–^1H COSY correlations. Furthermore, HMBC corre-

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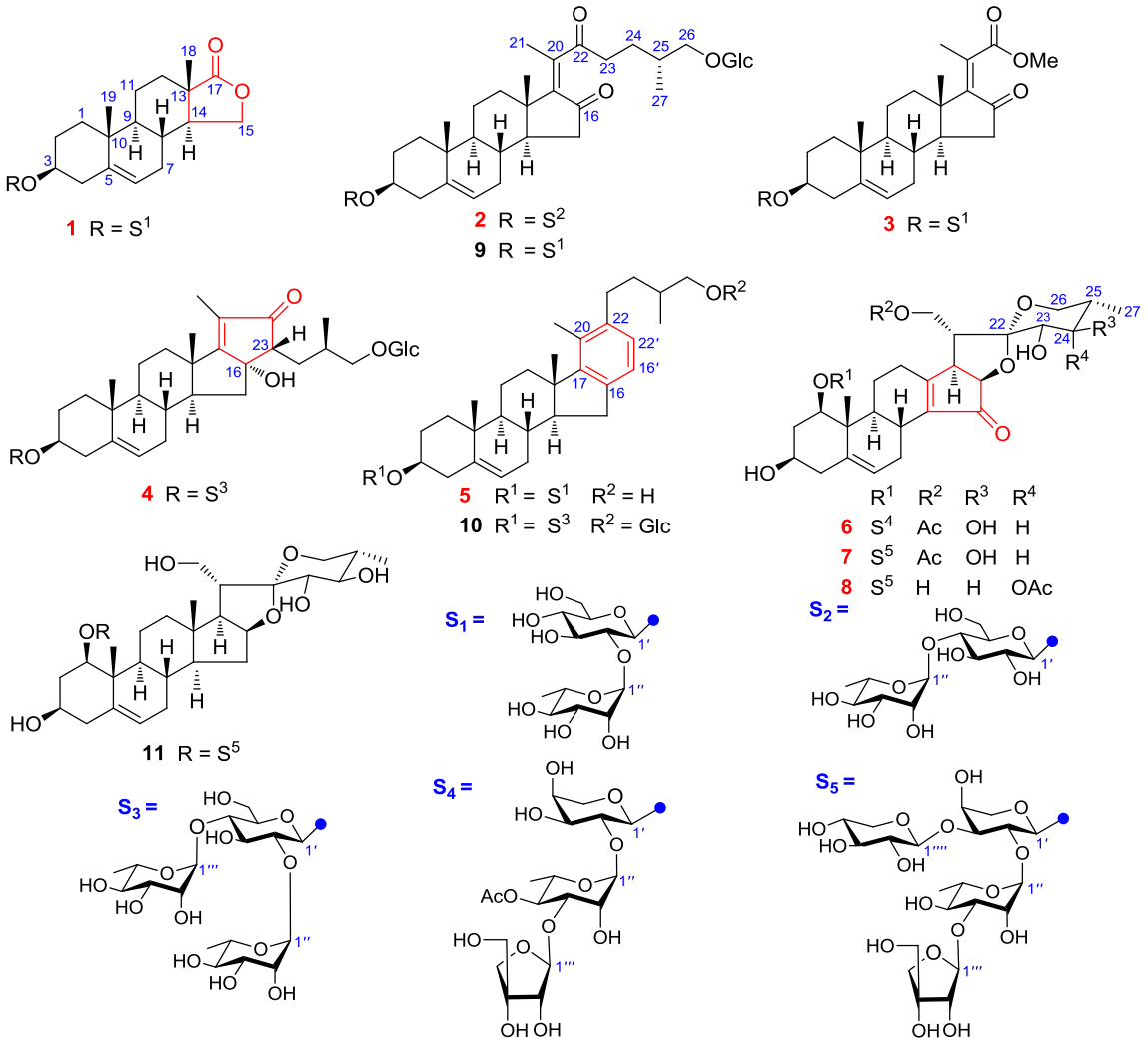

Fig. 1. Chemical structures of compounds **1**–**11** isolated from *T. kamtschaticum*.

lations from Me-19 to δ_C 37.3 (t, C-1), C-5, 49.8 (d, C-9), and 36.9 (s, C-10), from δ_H 2.79 (H-4a) to C-6, from Me-18 to δ_C 31.9 (t, C-12), 40.9 (s, C-13), 51.4 (d, C-14), and C-17, and from δ_C 4.18 (H-15a) to C-17 verified the connection of above substructural fragments as shown in Fig. 2. Based on these evidences, the aglycone of **1** was elucidated as 16-oxaandrost-5-en-3-ol-17-one. Although its analogue was ever synthesized by Banerjee & Gut,[12] the sapogenin featuring a rare skeleton was obtained in this study for the first time. The relative configurations of its aglycone were assigned by the following ROESY correlations: of Me-19 with δ_H 1.53 (H-8) and 1.66 (H-1a), of δ_H 0.88 (H-1b) with 3.90 (H-3), of Me-18 with H-15b, and of δ_H 0.86 (H-9) and H-15a with 1.69 (H-14). These ROESY cross-peaks also suggested the *trans*-fusion for the rings B/C and C/D, as well as the β-configuration for OH-3. Acid hydrolysis of **1** gave one D-glucose and one L-rhamnose, as determined by HPLC analysis (see Supplementary data S-2). The β-orientation of the anomeric proton of D-glucopyranosyl moiety was suggested by the large coupling constant ($^3J_{1,2}$ = 7.3 Hz), while the α-orientation for the L-rhamnopyranosyl unit was disclosed by its chemical shift values of C-3″ (δ_C 72.6) and C-5″ (δ_C 69.5) with those of the corresponding values of methyl α- and β-rhamnopyranoside.[13] In addition, the large $^1J_{C\text{-}1,H\text{-}1}$ value (172.5 Hz) for L-rhamnopyranosyl moiety also indicated that its anomeric proton shared an α-configuration.[14] Besides, HMBC correlations from δ_H 6.36 (Rha-H-1″) to δ_C 79.7 (Glc-C-2′) and from δ_H 5.02 (Glc-H-1′) to δ_C 77.8 (C-3) unambiguously assigned that the terminal rhamnopyranosyl unit was attached to C-2′ of the glucopyranosyl moiety and the saccharide chain was linked to C-3 of the aglycone. Therefore, structure **1** was elucidated as 16-oxaandrost-5-en-3-ol-17-one-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

Trillikamtoside L (**2**)[15] had a molecular formula of $C_{45}H_{70}O_{18}$, as deduced from HRESIMS at *m*/*z* 921.4470 $[M+Na]^+$ (calcd. for $C_{45}H_{70}O_{18}Na$, 921.4454) and ^{13}C NMR data. The IR spectrum displayed characteristic bands due to hydroxy (3424 cm^{-1}) and carbonyl (1712 cm^{-1}) functionalities, respectively. Its ^{13}C NMR spectrum displayed 45 carbon resonances, of which 27 belonged to the aglycone and the remaining18 ascribed to the sugar moiety. HMBC correlations from δ_H 1.95 (Me-21) to δ_C 205.6 (s, C-22), 145.6 (s, C-20), and 142.4 (s, C-17), from δ_H 0.92 (Me-18) to C-17, and from δ_H 2.10 (H-15a) to δ_C 50.3 (d, C-14) and 210.3 (s, C-16) indicated that two carbonyl groups were located at C-16 and C-22, respectively, as well as a double bond between C-17 and C-20. Additionally, HMBC correlations from Me-18 to δ_C 38.6 (t, C-12), 43.3 (s, C-13), and C-14, from δ_H 0.89 (Me-19 to δ_C 37.0 (t, C-1), 140.9 (s, C-5), 49.7 (d, C-9), and 36.9 (s, C-10), from δ_H 0.96 (Me-27) to δ_C 75.0 (t, C-26), C-24, and C-25, and from δ_H 2.11 (H-23a) to C-22, were observed. The aforementioned information, together with further analyses of ^1H–^1H COSY and ROESY experiments, established that the aglycone of **2** was dehydrokryptogenin.[3] The extensive analyses of 2D NMR spectroscopic data and the results of acid hydrolysis

Table 1
^{1}H NMR spectroscopic data for **1**–**5** in C_5D_5N (δ_H in ppm, *J* in Hz).

No.	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[b]
1a	1.66, m	1.66, m	1.69, m	1.76, m	1.70, m
1b	0.88, m	0.93, m	0.92, m	1.00, m	0.91, m
2a	2.11, m	2.04, m	2.13, m	2.08, m	2.11, m
2b	1.87, m	1.68, m	1.88, m	1.86, m	1.88, m
3	3.90, m	3.92, m	3.91, m	3.86, m	3.90, m
4a	2.79, m	2.80, br d (11.8)	2.78, dd (13.6, 4.7)	2.79, m	2.82, m
4b	2.71, m	2.68, dd (13.4, 2.3)	2.73, t (12.0)	2.74, m	2.76,m
6	5.22, br d (4.8)	5.26, br d (4.8)	5.25, br d (4.8)	5.33, br d (4.8)	5.31, br d (4.8)
7a	1.72, m	1.70, m	1.72, m	1.92, m	2.58, dd (14.5, 6.4)
7b	1.53, m	1.43, m	1.43, overlapped	1.55, m	2.38, m
8	1.53, m	1.45, m	1.43, overlapped	1.67, m	1.58, m
9	0.86, m	0.93, m	0.93, m	0.97, m	0.94, m
11a	1.44, m	1.49, m	1.49, m	1.54, m	1.53, 2H m
11b	1.33, m	1.42, m	1.42, m	1.45, m	
12a	1.84, m	2.76, 2H, d (7.5)	2.07, m	2.08, m	1. 89, m
12b	1.42, m		1.43, m	1.43, m	1.58, m
14	1.69, m	1.33, m	1.25, m	1.07, m	1.47, m
15a	4.18, dd (8.4, 6.8)	2.10, m	2.13, m	2.27, dd (12.9, 7.9)	2.11, m
15b	3.93, dd (11.6, 8.4)	1.49, t (11.3)	1.96, m	1.96, t (13.3)	1.88, m
18	0.95, s	0.92, s	0.84, s	1.44, s	0.90, s
19	0.98, s	0.89, s	1.03, s	1.06, s	1.08, s
21		1.95, s	2.03, s	1.82, s	2.31, s
23a		2.11, m		2.44, dd (10.3, 3.3)	1.94, m
23b		1.95, m			1.57, m
24a		2.08, overlapped		2.37, t (10.5)	1.93, m
24b		1.82, m		1.96, m	1.48, m
25		1.98, m		2.49, m	2.36, m
26a		3.92, m		4.02, t (8.1)	3.77, dd (10.4, 5.8)
26b		3.57, t (7.8)		3.71, dd (9.5. 6.3)	3.71, dd (10.4, 6.2)
27		0.96, d (6.6)		1.11, d (6.6)	1.11, d (6.6)
16′					7.10, d (7.6)
22′					7.05, d (7.6)
22-OMe			3.88, s		
Glc-1′	5.02, d (7.3)	4.94, d (7.8)	5.04, d (7.2)	4.94, d (7.2)	5.04, d (7.2)
2′	4.27, m	3.97, m	4.29, dd (5.1, 1.9)	4.22, overlapped	4.28, overlapped
3′	4.26, m	4.20, overlapped	4.28, br d (3.2)	4.22, overlapped	4.28, overlapped
4′	4.15, t (9.0)	4.43, t (9.3)	4.17, t (9.1)	3.65, br d (9.2)	4.17, t (9.2)
5′	3.93, dd (11.7, 8.4)	3.71, m	3.92, m	4.38, m	4.92, m
6′a	4.50, dd (9.6, 2.1)	4.24, m	4.52, dd (11.8, 2.1)	4.20, m	4.51, dd (11.9, 2.0)
6′b	4.34, overlapped	4.12, dd (11.7, 2.7)	4.36, overlapped	4.08, dd (11.5, 2.0)	4.35, m
Rha-1″	6.36, br s	5.87, br s	6.39, br s	6.39, br s	6.39, br s
2″	4.79, dd (3.2, 1.4)	4.68, br s	4.81, dd (3.3, 1.4)	4.84, m	4.81, m
3″	4.60, dd (9.5, 3.4)	4.56, dd (9.2, 3.1)	4.63, dd (9.2, 3.3)	4.63, dd (9.3, 3.1)	4.64, dd (9.3, 3.3)
4″	4.34, overlapped	4.33, m	4.36, overlapped	4.33, m	4.36, dd (9.3, 4.7)
5″	4.96, dq (9.4, 6.2)	4.99, m	5.00, m	4.96, m	5.01, m
6″	1.74, d (6.2)	1.69, d (6.1)	1.77, d (6.2)	1.76, d (6.2)	1.77, d (6.2)
Rha-1‴				5.85, br s	
2‴				4.68, br s	
3‴				4.54, dd (9.2, 3.3)	
4‴				4.36, m	
5‴				4.92, m	
6‴				1.61, d (6.1)	
Glc-1‴/1-⁗		4.94, d (7.8)		4.83, d (7.6)	
2‴/2⁗		4.00, m		4.02, t (8.1)	
3‴/3⁗		4.21, m		4.22, overlapped	
4‴/4⁗		4.20, overlapped		4.19, m	
5‴/5⁗		3.84, m		3.91, m	
6‴a/6⁗*a*		4.52, dd (11.8, 2.0)		4.52, dd (11.8, 2.0)	
6‴b/6⁗*b*		4.35, overlapped		4.34, m	

[a] Recorded at 500 MHz.
[b] Recorded at 600 MHz.

of **2** allowed for characterization of two *β*-D-glucopyranosyls and one α-L-rhamnopyranosyl. Comparison of the ^{1}H and ^{13}C NMR data attributable to the sugar portion with those of **1**, suggested that the terminal α-L-rhamnopyranosyl moiety present in **2** was linked to the C-4′ rather than that of C-2′ in **1**. This conclusion was supported by the HMBC correlations: δ_H 4.94 (Glc-H-1′) to δ_C 77.9 (d, C-3); δ_H 5.87 (Rha-H-1″) to δ_C 78.1 (d, Glc-C-4′); and δ_H 4.94 (d, Glc-H-1‴) to δ_C 75.0 (t, C-26). Thus, structure **2** was defined as 26-*O*-*β*-D-glucopyranosyl-17(20)-dehydrokryptogenin -3-*O*-α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranoside.

Trillikamtoside M (**3**)[16] had a molecular formula of $C_{35}H_{52}O_{13}$ based on its HRESIMS (*m*/*z* 681.3488 $[M+H]^+$) and ^{13}C NMR data. Comparison ^{13}C NMR data of **3** with those of **2** suggested that they were analogues, except for the presence of a methoxy group [δ_H 3.88, δ_C 52.2] and the absence of the signals for the side chain of C-23–C-27 in the aglycone of 17(20)-dehydrokryptogenin. This observation indicated that the aglycone of **3** showed be a split derivative of **2** between C-22 and C-23, which was further certified by the HMBC correlations from δ_H 2.03 (Me-21) to δ_C 144.9 (s, C-17) and 172.2 (s, C-22) and from δ_H 3.88 (CO<u>OMe</u>) to C-22.

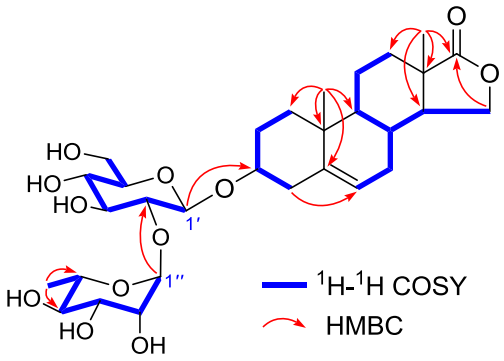

Fig. 2. The ^{1}H–^{1}H COSY and key HMBC correlations of **1**.

The sugar sequence of **2** was in good agreement with those of saponins **1** and **9** by comparison of their ^{1}H and ^{13}C NMR data. This was unambiguously verified by HMBC correlations from δ_H 5.04 (Glc-H-1′) to δ_C 77.8 (d, C-3) and from δ_H 6.39 (Rha-H-1″) to δ_C 79.7 (d, Glc-C-2′). Consequently, structure **3** was defined as pregna-5,17(20)-diene-20-carboxylic acid methyl ester-3-ol-16-one-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

Trillikamtoside N (**4**)[17] was assigned the molecular formula of $C_{51}H_{80}O_{22}$ in accordance with its HRESIMS (*m*/*z* 1067.5034 [M+Na]$^+$) and ^{13}C NMR data. The IR absorptions at 3424 cm^{-1} and 1702 cm^{-1} were indicative of hydroxy and α,β-unsaturated ketone groups, respectively. The ^{13}C NMR spectroscopic data of **4** exhibited characteristic signals ascribed to one carbonyl at δ_C 212.4 (s, C-22), four olefinic carbons at δ_C182.2 (s, C-17), 141.0 (s, C-5), 128.2 (s, C-20), and 121.7 (d, C-6), an oxygenated quaternary carbon at δ_C 83.0 (s, C-16), as well as four methyls at δ_C 15.7 (q, Me-18), 19.5 (q, Me-19), 8.6 (q, Me-21), and 17.2 (q, Me-27). The aforementioned NMR data (Table 2) suggested that **4** was a cholestanol saponin as that of ypsiyunnoside A[18] with the main difference of the absence of one α-L-rhamnopyranosyl unit. The sequence of sugar chain attached to C-3 in **4** was identical with that of **10** by comparison of their ^{1}H and ^{13}C NMR data (Tables 1 and 2) together with results of acid hydrolysis. Accordingly, structure **4** was established as 26-*O*-β-D-glucopyranosyl-(23*R*,25*R*)-16,23-cyclocholest-5,17(20)-dien-22-one-3,16,26-triol-3-*O*-α-L-rhamnopyranosyl-(1–4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside.

Trillikamtoside O (**5**)[19] gave a molecular formula of $C_{41}H_{62}O_{11}$ by an ion peak at *m*/*z* 753.4194 [M+Na]$^+$ in the HRESIMS and ^{13}C NMR data. The ^{13}C NMR spectroscopic data (Table 2) in the downfield portion of **5** showed a series of benzene ring signals: δ_C 152.3 (s, C-17), 141.5 (s, C-16), 140.5 (s, C-22), 131.7 (s, C-20), 127.9 (d, C-22′), and 123.4 (d, C-16′). Detailed analysis of the HSQC, HMBC, ^{1}H–^{1}H COSY, and ROESY experiments indicated that it had a aglycone of homo-cholestane same as that of aethioside A (**10**).[10] The sugar chain placed at C-3 of **5** was identified the same as those of **1** and **3** by comparing their ^{1}H and ^{13}C NMR data (Tables 1 and 2) together with results of acid hydrolysis. Hence, structure **5** was established as 26-hydroxyl-homo-aro-cholest-5-en-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

Trillikamtoside P (**6**)[20] was assigned the molecular formula of $C_{46}H_{66}O_{23}$ from its HRESIMS (*m*/*z* 993.3942 [M+Na]$^+$ and ^{13}C NMR data. The IR spectrum showed absorptions bands for hydroxy, α,β-unsaturated carbonyl, and olefinic groups at 3432, 1732, 1701, and 1626 cm^{-1}, respectively. In the ^{1}H NMR spectrum (Table 3), one tertiary methyl at δ_H 1.21 (3H, s) and two secondary methyls at δ_H 1.01 (3H, d, *J* = 6.3 Hz) and 1.45 (3H, d, *J* = 6.0 Hz) were observed, as well as three anomeric protons at δ_H 6.28 (1H, br s),

Table 2
^{13}C NMR spectroscopic data for **1**–**5** in C_5D_5N.

No.	1[a]	2[b]	3[a]	4[a]	5[b]	Position	1[a]	2[b]	3[a]	4[a]	5[b]
1	37.3, t	37.0, t	37.2, t	37.6, t	37.8, t	22-OMe			52.2, q		
2	30.1, t	30.0, t	30.2, t	30.2, t	30.7, t	3-*O*-Glc					
3	77.8, d	77.9, d	77.8, d	78.1, d	78.9, d	1′	100.4, d	102.4, d	100.4, d	100.4, d	100.8, d
4	39.0, t	39.1, t	38.9, t	39.0, t	39.5, t	2′	79.7, d	75.4, d	79.7, d	78.1, d	80.2, d
5	141.3, s	140.9, s	141.0, s	141.0, s	141.2, s	3′	77.8, d	76.6, d	77.8, d	77.9, d	78.3, d
6	120.7, d	121.2, d	121.3, d	121.7, d	122.3, d	4′	71.8, d	78.1, d	71.8, d	78.7, d	72.3, d
7	30.8, t	31.5, t	31.7, t	31.9, t	32.9, t	5′	78.4, d	77.1, d	78.4, d	77.1, d	78.3, d
8	29.0, d	30.6, d	30.7, d	31.9, d	31.4, d	6′	62.7, t	61.4, t	62.7, t	61.4, t	63.1, t
9	49.8, d	49.7, d	49.8, d	50.6, d	50.9, d	2′-*O*-Rha					
10	36.9, s	36.9, s	37.1, s	37.3, s	37.6, s	1″	102.1, d		102.1, d	102.2, d	102.6, d
11	20.1, t	20.8, t	20.8, t	20.7, t	21.7, t	2″	72.6, d		72.6, d	72.6, d	73.1, d
12	31.9, t	38.6, t	35.9, t	35.8, t	37.4, t	3″	72.9, d		72.9, d	72.8, d	73.4, d
13	40.9s	43.3, s	43.6, s	44.1, s	47.6, s	4″	74.2, d		74.2, d	74.0, d	74.7, d
14	51.4, d	50.3, d	49.9, d	53.8, d	58.1, d	5″	69.5, d		69.5, d	69.7, d	70.0, d
15	69.1, t	35.9, t	38.0, t	38.5, t	32.0, t	6″	18.7, q		18.7, q	18.8, q	19.2, q
16		210.3, s	204.4, s	83.0, s	141.5, s	4′-*O*-Rha					
17	181.2, s	142.4, s	144.9, s	182.2, s	152.3, s	1‴		102.6, d		103.0, d	
18	13.9, q	16.8, q	16.5, q	15.7, q	17.0, q	2‴		72.5, d		72.7, d	
19	19.3, q	19.2, q	19.3, q	19.5, q	19.8, q	3‴		72.7, d		73.0, d	
20		145.6, s	134.2, s	128.2, s	131.7, s	4‴		73.9, d		74.2, d	
21		15.6, q	16.1, q	8.6, q	15.2, q	5‴		70.3, d		70.5, d	
22		205.6, s	172.2, s	212.4, s	140.5, s	6‴		18.5, q		18.6, q	
23		37.8, t		57.5, d	32.5, t	26-*O*-Glc					
24		27.8, t		29.4, t	36.0, t	1⁗		104.8, d		105.4, d	
25		33.3, d		32.1, d	37.2, d	2⁗		75.1, d		75.4, d	
26		75.0, t		76.7, t	67.9, t	3⁗		78.5, d		78.6, d	
27		17.3, q		17.2, q	17.8, q	4⁗		71.6, d		71.9, d	
16′					123.4, d	5⁗		78.4, d		78.7, d	
22′					127.9, d	6⁗		62.7, t		62.9, t	

[a] Recorded at 125 MHz.
[b] Recorded at 150 MHz.

Table 3
^{1}H and ^{13}C NMR spectroscopic data for **6**–**8** in C_5D_5N.

No.	**6**[a]		**7**[b]		**8**[c]	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	3.71, dd (11.9, 2.7)	84.7, d	3.70, dd (11.9, 3.9)	85.0, d	3.70, m	84.6, d
2a	2.61, overlapped	38.0, t	2.65, overlapped	37.9, t	2.65, m	37.7, t
2b	2.35, q (11.8)		2.47, q (11.9)		2.44, q (11.8)	
3	3.85, m	68.0, d	3.82, m	68.4, d	3.81, m	68.3, d
4a	2.61, overlapped	43.2, t	2.58, t (11.2)	43.3, t	2.57, m	43.3, t
4b	2.58, m		2.51, dd (12.5, 4.7)		2.51, m	
5		139.7, s		139.8, s		139.4, s
6	5.62, br d (4.8)	125.1, d	5.57, br d (4.8)	125.0, d	5.57, br d (4.8)	125.0, d
7a	3.26, m	29.5, t	3.22, m	29.6, t	3.23, m	29.6, t
7b	1.67, m		1.65, m		1.64, m	
8	2.23, m	32.0, d	2.18, br t (11.7)	32.0, d	2.10, m	32.0, d
9	1.70, m	47.8, d	1.69, br t (11.8)	47.9, d	1.66, m	47.8, d
10		42.4, s		42.5, s		42.6, s
11a	3.22, m	25.3, t	3.24, m	25.4, t	3.08, m	25.3, t
11b	1.04, m		1.02, overlapped		0.85, m	
12a	2.67, m	28.1, t	2.65, overlapped	28.2, t	2.61, m	28.2, t
12b	2.61, overlapped		2.61, m		2.55, m	
13		175.5, s		175.7, s		176.6, s
14		139.2, s		139.4, s		138.8, s
15		203.7, s		203.7, s		204.4, s
16	4.74, d (5.3)	81.3, d	4.73, d (5.4)	81.3, d	4.73, d (6.5)	81.9, d
17	3.21, m	45.4, d	3.19, overlapped	45.4, d	3.18, t (7.4)	48.4, d
19	1.21, s	13.9, q	1.24, s	14.1, q	1.18, s	14.0, q
20	3.19, m	49.3, d	3.19, overlapped	49.2, d	2.99, q (6.5)	49.9, d
21a	4.53, m	64.2, t	4.56, dd (10.7, 6.9)	64.2, t	4.28, m	61.8, t
21b	4.50, m		4.50, dd (10.7, 6.4)		4.15, m	
22		114.0, s		114.0, s		112.4, s
23	4.00, m	74.4, d	3.98, m	74.5, d	4.48, br s	68.9, d
24	3.99, m	75.2, d	3.96, m	75.3, d	5.72, t (3.0)	73.2, d
25	1.99, m	39.1, d	1.98, m	39.2, d	2.02, m	34.6, d
26a	3.66, m	65.2, t	3.65, m	65.2, t	4.01, t (11.8)	61.6, t
26b	3.58, m		3.56, m		3.39, dd (11.8, 4.4)	
27	1.01, d (6.3)	13.3, q	1.02, d (6.5)	13.4, q	0.74, d (6.8)	12.4, q
Ara-1′	4.54, d (7.6)	100.9, d	4.57, d (7.2)	101.1, d	4.56, d (7.2)	100.9, d
2′	4.11, m	75.9, d	4.57, overlapped	73.4, d	4.57, m	73.5, d
3′	4.48, m	74.7, d	4.04, m	85.0, d	4.04, m	84.9, d
4′	4.12, m	70.3, d	4.39, m	69.8, d	4.40, m	69.9, d
5′a	4.21, br d (12.4)	67.7, t	4.18, br d (11.6)	67.2, t	4.19, (11.6)	67.2, t
5′b	3.64, br d (11.8)		3.67, br d (12.2)		3.67, (12.2)	
Rha-1″	6.28, br s	101.1, d	6.37, br s	101.5, d	6.38, br s	101.5, d
2″	4.87, br s	71.7, d	4.95, br s	71.9, d	4.96, br s	71.9, d
3″	4.70, dd (9.8, 2.3)	78.3, d	4.63, dd (9.4, 2.7)	80.2, d	4.64, m	80.0, d
4″	5.91, t (10.0)	74.4, d	4.38, m	72.6, d	4.39, m	72.6, d
5″	4.94, m	66.8, d	4.85, m	69.5, d	4.83, overlapped	69.5, d
6″	1.45, d (5.9)	18.4, q	1.67, d (6.0)	19.2, q	1.66, d (6.1)	19.2, q
Api-1‴	5.89, d (2.8)	112.4, d	6.21, d (2.5)	111.9, d	6.22, d (2.5)	111.9, d
2‴	4.64, d (2.8)	77.8, d	4.83, d (2.5)	77.9, d	4.83, overlapped	77.8, d
3‴		80.0, s		80.3, s		80.3, s
4‴*a*	4.55, d (9.4)	75.0, t	4.62, d (9.3)	75.2, t	4.62, d (9.3)	75.2, t
4‴*b*	4.26, d (9.4)		4.26, d (9.3)		4.26, d (9.3)	
5‴	4.01, 2H br s	65.3, t	4.14, 2H d (7.9)	65.6, t	4.14, 2H d (7.4)	65.7, t
Xyl-1⁗			4.96, d (7.8)	106.8, d	4.97, d (7.7)	106.8, d
2⁗			3.86, t (7.9)	74.7, d	3.90, t (7.9)	74.8, d
3⁗			4.08, overlapped	78.6, d	4.10, overlapped	78.6, d
4⁗			4.08, overlapped	71.0, d	4.10, overlapped	70.1, d
5⁗a			4.26, m	67.2, t	4.28, m	67.2, t
5⁗b			3.66, m		3.67, m	
21-MeCO–	2.01, s	20.9, q	2.05, s	21.1, q		
21-MeCO–		170.8, s		171.1s		
24-MeCO–					1.89, s	21.1, q
24-MeCO–						171.6, s
4″-MeCO–	2.16, s	21.0, q				
4″-MeCO–		171.0, s				

[a] Recorded at 400 and 100 MHz.
[b] Recorded at 500 and 125 MHz.
[c] Recorded at 600 and 150 MHz.

5.89 (1H, d, *J* = 2.8 Hz), and 4.54 (1H, d, *J* = 7.6 Hz). The ^{13}C NMR (Table 3) and HSQC spectra exhibited the presence of 46 carbon resonances, 26 of which were assigned to the aglycone moiety comprising two methyls, seven methylenes (two oxygenated ones), ten methines (five oxygenated ones and a olefinic one), four quaternary carbons (three olefinic ones), a keto-carbonyl and a ketal carbons, while the remaining signals were due two acetyls and the three sugar units.

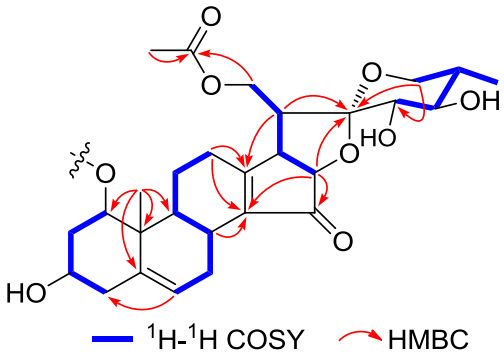

Fig. 3. Key ^{1}H–^{1}H and HMBC correlations of the aglycone moiety of **6**.

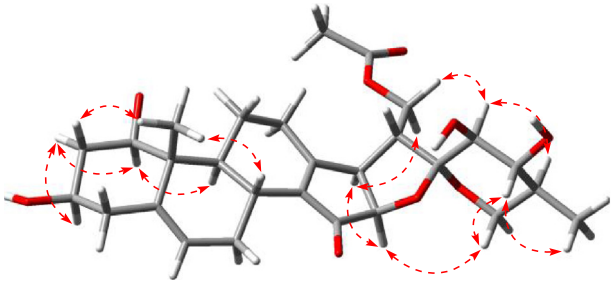

Fig. 4. Key ROESY correlations of the aglycone moiety of **6**.

Detailed inspection of the 1D and 2D NMR (including ^{1}H–^{1}H COSY, HSQC, HMBC, and ROESY) spectral data (Figs. 3 and 4) of **6** established that its aglycone was trillenogenin.[21] However, acid hydrolysis of **6** afforded D-apiose, L-rhamnose, and L-arabinose. It is noted that one acetyl group was substantiated to be placed at C-4″ of L-the rhamnopyranosyl moiety based on the HMBC correlations from δ_H 5.91 (H-4″) and 2.16 (3H, s, MeCO–) to δ_C 171.0 (MeCO–). The β-orientation for the D-apiofuranosyl was determined by comparing its chemical shifts of δ_C 112.4 (d, C-1‴), 77.8 (d, C-2‴), 80.0 (s, C-3‴), 75.0 (t, C-4‴), and 65.3 (t, C-5‴) with those of corresponding carbons of α- and β-D-apiofuranoside,[22,23] while the large coupling constant ($^3J_{1,2}$ > 7.0 Hz) of the anomeric proton for the L-arabinopyranosyl suggested that it was α-orientated. Finally, the connectivity between the trisaccharide chain and the aglycone was inferred by the following HMBC correlations from δ_H 4.54 (Ara-H-1′) to δ_C 84.7 (d, C-1), from δ_H 6.28 (Rha-H-1″) to δ_C 75.9 (d, Ara-C-2′), and from δ_H 5.89 (Api-H-1‴) to δ_C 78.3 (d, Rha-C-3″). On the basis of above evidence, structure **6** was deduced as 21-*O*-acetyl-trillenogenin-1-*O*-β-D-apiofuranosyl-(1→3)-4″-acetyl-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

Trillikamtoside Q (**7**)[24] was shown to have a molecular of $C_{49}H_{72}O_{25}$ by HRESIMS (*m*/*z* 1083.4248 [M+Na]$^+$) and ^{13}C NMR spectra. Careful comparison of NMR (Table 3) spectroscopic data of **7** with those of **6** indicated they shared the same aglycone except for the signals of the sugar moiety. The ^{1}H NMR spectrum of **7** exhibited four anomeric protons at δ_H 6.37 (br s), 6.21 (d, *J* = 2.5 Hz), 4.96 (d, *J* = 7.8 Hz), and 4.57 (d, *J* = 7.2 Hz), which were HSQC-correlated with δ_C 101.5, 111.9, 106.8, and 101.1, respectively. These signals suggested **7** contained D-apiose, L-rhamnose, D-xylose, and L-arabinose moieties, which was supported by the results of sugar analysis. The coupling constant ($^3J_{1,2}$ > 7.0 Hz) of the anomeric proton for the additional D-xylopyranosyl suggested that it was β-configuration. The sequence of the four sugars and the linkage site to the aglycone were assigned by the HMBC correlations: from δ_H 4.57 (Ara-H-1′) to δ_C 85.0 (d, C-1), from δ_H 6.37 (Rha-H-1″) to δ_C 73.4 (d, Ara-C-2′), from δ_H 6.21 (Api-H-1‴) to δ_C 80.2 (Rha-C-3″), and fromδ_H 4.96 (Xyl-H-1‴') to δ_C 85.0 (Ara-C-3′). Structure **7** was thus established as 21-*O*-acetyl-trillenogenin-1-*O*-β-D-apiofuranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside.

Table 4
Cytotoxicities (IC_{50}, μM) of the isolates from *T. kamtschaticum*.[a]

Compounds	HCT116	Compounds	HCT116
2	17.28 ± 2.69	**8**	5.84 ± 1.05
6	4.92 ± 1.00	**9**	12.70 ± 1.18
7	22.48 ± 8.68	CPT	0.0115 ± 0.0009

[b]Positive control.
[a] Other compounds were inactive (IC_{50} > 40 μM).

Trillikamtoside R (**8**)[25] had the same molecular formula of $C_{49}H_{72}O_{25}$ as that of **7** by HRESIMS (*m*/*z* 1083.4249 [M+Na]$^+$) and ^{13}C NMR data. Inspection of the ^{1}H and ^{13}C NMR spectroscopic data (Table 3) of **8** with those of **7** revealed that the acetyl was attached to C-24 in **8** instead of that C-21 in **7**. This conclusion was confirmed by the HMBC correlation from δ_H 5.72 (H-24) and 1.89 (MeCO–) to δ_C 171.6 (MeCO–). Additionally, ROESY correlation of δ_H 4.48 (H-23) with H-24 determined α-configuration for OH-24. According to the literature,[26] the aglycone of **8** was defined as 24-*O*-acetyl-epitrillengenin. The saccharide moiety of **8** was consistent with that of **7** by the further analysis of ^{1}H–^{1}H COSY, HSQC, and HMBC experiments. Structure **8** was therefore elucidated as 24-*O*-acetyl-epitrillengenin-1-*O*-β-D-apiofuranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside.

All isolated saponins were evaluated for their cytotoxicities against human colorectal cancer cells (HCT116) by MTT assay (see Supplementary data S-4).[27] The results were summarized in Table 4. It could be found that the new compound **6** showed the strongest cytotoxic activity with an IC_{50} value of 4.92 μM. Compounds **2** and **7**–**9** also exhibited inhibitory effects with IC_{50} values of 17.28, 22.48, 5.84, and 12.70 μM, respectively. Whereas, other compounds with IC_{50} values higher than 40 μM were inactive. As indicated by the IC_{50} values of **6** and **7**, which had the same aglycone (21-*O*-acetyl-trillenogenin), the introduction of D-Xylose to the inner L-Arabinose could decrease the cytotoxicity. Although compounds **7** and **8** shared the same saccharide moiety, the transformation of configuration of OH-24 and the transference of the acetyl group from C-21 to C-24 could evidently strengthen the cytotoxicity of the latter one.

In summary, eleven steroidal glycosides including eight new structures (**1**–**8**) were isolated from the 30% EtOH elution from a macroporous resin of the crude extract of *T. kamtschaticum*. The aglycone of saponin **1**, 16-oxaandrost-5-en-3-ol-17-one, is reported as a natural occurrence for the first time. More importantly, **6** and **8** showed good cytotoxicity against HCT116 cells with IC_{50} values of 4.92 and 5.84 μM, respectively.

Acknowledgments

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.04.057.

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9. The air-dried and powdered plant material (10 kg) was extracted three times with 75% EtOH (20 L, each) under reflux for a total of 6 h (3 × 2 h) and d then removed solvents under reduced pressure to give a crude extract . This extract was suspended in water and partitioned with *n*-butyl alcohol to afford total saponins moiety after removal of the solvents *in vacuo*. The *n*-butyl extract (1.2 kg) was subjected to D101 macroporous resin eluted successively with aqueous EtOH gradient system (0%, 30%, 70%, and 95%). The 30% EtOH fraction (430.5 g) was passed through a silica gel (300−400 mesh) with gradients of $CHCl_3$-MeOH-H_2O (10:1:0→2:1:0.5, v/v) to give four fractions (Fr. 1→Fr. 4). Fr. 1 (97.8 g) was further subjected to a RP-18 column and eluted with MeOH-H_2O (20:80→60:40, v/v) to give five fractions (Fr.1.1→Fr.1.5). Fr. 1.1 (19.8 g) and Fr. 1.3 (10.3 g) were chromatographed on Sephadex LH-20 ($CHCl_3$-MeOH, 1:1, v/v), followed by semi-preparative HPLC to yield **1** (42.4 mg), **3** (27.4 mg), **4** (15.8 mg), and **5** (3.2 mg). Fr.1.5 (16.3 g) were subjected to a RP-18 column chromatography (MeOH-H_2O, 30:70→40:60, v/v) to yield **10** (1.5 g). Fr.3 (33.3 g) was purified repeatedly by a silica gel CC ($CHCl_3$-MeOH-H_2O, 10:1:0→7:3:0.5, v/v) to afford **2** (27.4 mg), and **9** (1.3 g), respectively. Fr. 4 (39.5 g) was separated by silica gel CC ($CHCl_3$-MeOH-H_2O, 10:1:0→7:3:0.5, v/v) to give three subfractions. Fr. 4.1 (3.5 g) was separated by semi-prep. HPLC (MeCN–H_2O, 20:80→50:50, v/v) to yield **6** (100.0 mg), **7** (7.6 mg), and **8** (20.0 mg). Fr. 4.3 (8.8 g) was subjected to a RP-18 column chromatography (MeOH-H_2O, 20:70→50:50, v/v) to give **11**(1.0 g).
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15. Trillikamtoside L (**2**): White amorphous powder; $[\alpha]_D^{18}$ −64.1 (*c* 0.15, MeOH); IR (KBr) ν_{max} 3424, 2933, 1712, 1630, 1383 cm^{-1}; HRESIMS *m/z*: 921.4470 $[M+Na]^+$ (calcd. for $C_{45}H_{70}O_{18}Na$, 921.4454); ^1H and ^{13}C NMR data, see Tables 1 and 2.
16. Trillikamtoside M (**3**): White amorphous powder; $[\alpha]_D^{18}$ −59.9 (*c* 0.22, MeOH); IR (KBr) ν_{max} 3429, 2935, 1724, 1636, 1382, 1291 cm^{-1}; HRESIMS *m/z*: 681.3488 $[M+H]^+$ (calcd. for $C_{35}H_{53}O_{14}$, 681.3481); ^1H and ^{13}C NMR data, see Tables 1 and 2.
17. Trillikamtoside N (**4**): White amorphous powder; $[\alpha]_D^{18}$ −108.0 (*c* 0.15, MeOH); IR (KBr) ν_{max} 3424, 2932, 1702, 1658, 1382 cm^{-1}; HRESIMS *m/z*: 1067.5034 $[M+Na]^+$ (calcd for $C_{51}H_{80}O_{22}Na$, 1067.5033); ^1H and ^{13}C NMR data, see Tables 1 and 2.
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19. Trillikamtoside O (**5**): White amorphous powder; $[\alpha]_D^{18}$ −111.8 (*c* 0.07, MeOH); IR (KBr) ν_{max} 3440, 2931, 1630, 1383 cm^{-1}; HRESIMS *m/z*: 753.4194 $[M+Na]^+$ (calcd. for $C_{41}H_{62}O_{11}Na$, 753.4190); ^1H and ^{13}C NMR data, see Tables 1 and 2.
20. Trillikamtoside P (**6**): White amorphous powder; $[\alpha]_D^{18}$ −101.1 (*c* 0.36, MeOH); IR (KBr) ν_{max} 3432, 2935, 1733, 1701, 1626 cm^{-1}; HRESIMS *m/z*: 993.3942 $[M+Na]^+$ (calcd. for $C_{46}H_{66}O_{22}Na$, 993.3943); ^1H and ^{13}C NMR data, see Table 3.
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24. Trillikamtoside Q (**7**): White amorphous powder; $[\alpha]_D^{18}$ −83.3 (*c* 0.33, MeOH); IR (KBr) ν_{max} 3424, 2930, 1700, 1628, 1384, 1251 cm^{-1}; HRESIMS *m/z*: 1083.4248 $[M+Na]^+$ (calcd. for $C_{49}H_{72}O_{25}Na$, 1083.4255); ^1H and ^{13}C NMR data, see Table 3.
25. Trillikamtoside R (**8**): White amorphous powder; $[\alpha]_D^{18}$ −86.4 (*c* 0.15, MeOH); IR (KBr) ν_{max} 3418, 2930, 1703, 1628, 1384, 1255 cm^{-1}; HRESIMS *m/z*: 1083.4249 $[M+Na]^+$ (calcd. for $C_{49}H_{72}O_{25}$, 1083.4255); ^1H and ^{13}C NMR data, see Table 3.
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