Two New Steroid Glycosides and a New Sesquiterpenoid Glycoside from the Underground Parts of *Trillium kamtschaticum*

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Two new steroid glycosides, named trikamsterosides A and B, and a new sesquiterpenoid glycoside named trikamsesuquiside A were isolated from the underground parts of *Trillium kamtschaticum* PALL. along with 18 known compounds comprising 12 steroids, one sesquiterpenoid glycoside, one phenylpropanoid, one flavonoid glycoside, and three phenylpropanoid sucrose esters. Their chemical structures were determined on the basis of spectroscopic data and chemical evidence. Among them, one phenylpropanoid sucrose ester showed almost the same radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl as that of α-tocopherol.

Key words *Trillium kamtschaticum*; steroid glycoside; sesquiterpenoid glycoside; phenylpropanoid sucrose ester; radical-scavenging effect

Trillium kamtschaticum PALL. is a liliaceous plant that grows wild in Northeast Asia. With regard to the chemical constituents of the underground parts of this plant, one of the present authors reported the isolation and structural elucidation of four novel steroid glycosides 18-norspirostanol derivatives.[1—3] However, their detailed NMR spectral data had not been described. In the preceding paper, we reported the isolation of 11 steroids including six 18-norspirostanol derivatives from the underground parts of *T. kamtschaticum* and detailed ^{1}H- and ^{13}C-NMR spectral data of the 18-norspirostanol derivatives.[4] As part of our continuing study of this plant, we describe herein the isolation and structural elucidation of two new steroid glycosides and a new sesquiterpenoid glycoside along with 18 known compounds comprising 12 steroids, one sesquiterpenoid glycoside, one phenylpropanoid, one flavonoid glycoside, and three phenylpropanoid sucrose esters from the MeOH extract of the underground parts as well as the radical-scavenging effects of the five phenolic compounds.

The MeOH extract of the underground parts of *T. kamtschaticum* was successively subjected to Diaion HP20, silica gel, and Chromatorex ODS column chromatography as well as HPLC on ODS to afford 21 compounds (**1**—**21**).

Compounds **5**—**21** were identified as β-ecdysone (**5**),[5] polypodine B (**6**),[5] penogenin 3-*O*-β-D-glucopyranoside (**7**),[6] penogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside (**8**),[6] penogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-[*O*-α-L-rhamnopyranosyl-(1→4)]-*O*-β-D-glucopyranoside (**9**),[6,7] penogenin 3-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→4)-[*O*-α-L-rhamnopyranosyl-(1→2)]-*O*-β-D-glucopyranoside (**10**),[6] deoxytrillenoside (**11**),[3,4] 26-*O*-β-D-glucopyranosyl (22Ψ,25*R*)-furost-5-ene-3β,17α,22,26-tetraol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside (**12**),[6] 26-*O*-β-D-glucopyranosyl (22Ψ,25*R*)-furost-5-ene-3β,17α,22,26-tetraol 3-*O*-α-L-rhamnopyranosyl-(1→2)-[*O*-α-L-rhamnopyranosyl-(1→4)]-*O*-β-D-glucopyranoside (**13**),[3,8] 26-*O*-β-D-glucopyranosyl 17(20)-dehydrokryptogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside (**14**),[6] 26-*O*-β-D-glucopyranosyl 17(20)-dehydrokryptogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-[*O*-α-L-rhamnopyranosyl-(1→4)]-*O*-β-D-glucopyranoside (**15**),[7] 7,11-dimethyl-3-methylene-1,6-dodecadien-10,11-diol 10-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranoside (**16**),[9] methyl ferulorate (**17**),[10] astragalin (**18**),[11] 3-*O*-feruloylsucrose (**19**),[12] regaloside A (**20**),[10] and heronioside A (**21**)[10] based on their physical and spectral data, although the detailed ^{13}C-NMR data of **7**, **8**, **10**, **12**, and **14** and ^{1}H- and ^{13}C-NMR assignments of **16** have not been reported.

Compound **1** was obtained as an amorphous powder. In the positive FAB-MS, **1** indicated an $[M+Na]^+$ ion peak at *m*/*z* 777. The molecular formula of **1** was determined as $C_{39}H_{62}O_{14}$ by high-resolution (HR) positive FAB-MS. The ^{1}H-NMR spectrum of **1** showed signals due to two tertiary methyl groups (δ 1.10, 0.98), two secondary methyl groups [δ 1.78 (d, $J=6.0$ Hz), 1.26 (d, $J=7.5$ Hz)], one olefinic proton [δ 5.30 (d, $J=5.5$ Hz)], and two monosaccharide groups, one of which was considered a rhamnosyl group. The ^{13}C-NMR spectrum of **1** was similar to that of **8** apart from the appearance of the signal due to one oxygenated methylene carbon and the lack of signal due to one methyl carbon. These ^{1}H- and ^{13}C-NMR signals (Tables 1, 2, 3) were assigned with the aid of ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC) techniques, and **1** was believed to be a 27-hydroxylated derivative of **8**. Furthermore, the coupling constant values of the signals [δ 4.08 (dd, $J=3.5$, 11.0 Hz), 3.91 (dd, $J=11.0$, 11.0 Hz)] due to H_2-26 indicated the hydroxylmethyl group at C-25 to exhibit *equatorial*-orientation. Consequently, **1** was elucidated as (25*S*)-27-hydroxypenogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside (Fig. 1), which has been reported as a constituent of the rhizome of *T. erectum*, but the physical and spectral data were not previously described.[13]

Compound **2**, named trikamsteroside A, was obtained as an amorphous powder, and HR-positive FAB-MS indicated the molecular formula of **2** to be the same as that of **1**. The ^{1}H- and ^{13}C-NMR spectra, which were assigned by tech-

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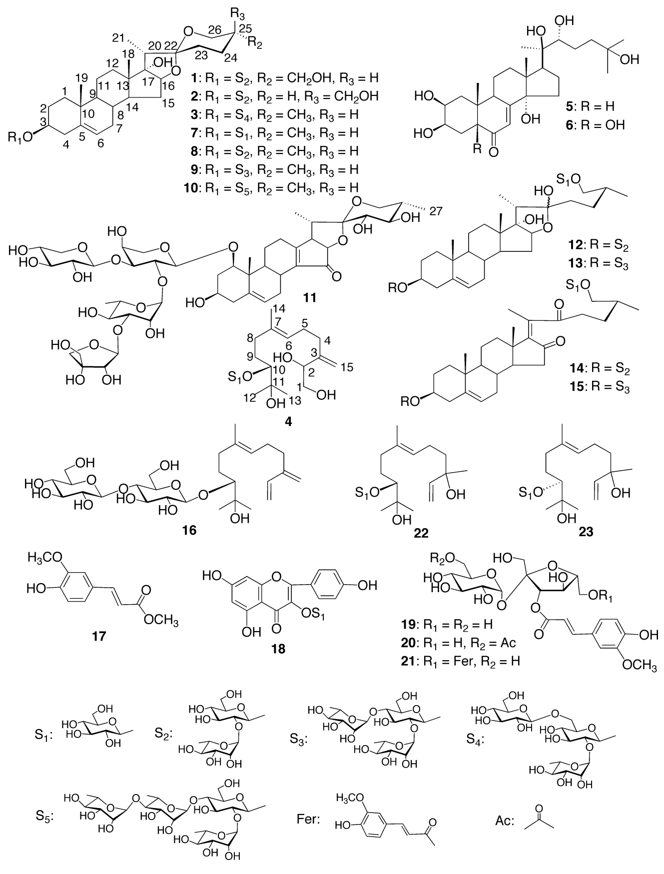

Fig. 1. Structures of **1**—**23**

Table 1. ^{1}H-NMR Data for **1**—**3** (in C_5D_5N, 500 MHz)

H	**1**	**2**	**3**
3	*ca.* 3.94	*ca.* 3.94	*ca.* 3.95
4a	2.81 dd (5.0, 13.0)	2.81 dd (5.0, 13.0)	*ca.* 2.78
4b	2.76 dd (13.0, 13.0)	2.76 dd (13.0, 13.0)	*ca.* 2.78
6	5.30 d (5.5)	5.31 d (5.0)	5.28 d (4.5)
16	4.50 dd (7.0, 7.0)	4.46 dd (7.5, 7.5)	4.47 dd (7.0, 7.0)
18	0.98 s	0.95 s	0.96 s
19	1.10 s	1.10 s	1.09 s
20	2.39 q (7.5)	2.23 q (7.5)	2.28 q (7.0)
21	1.26 d (7.5)	1.20 d (7.5)	1.24 d (7.0)
26a	4.08 dd (3.5, 11.0)	4.14 dd (2.0, 11.0)	*ca.* 3.52
26b	3.91 dd (11.0, 11.0)	3.94 br d (11.0)	*ca.* 3.52
27a	3.74 dd (5.0, 10.5)	4.14 dd (2.0, 11.0)	0.70 d (5.5)
27b	3.65 dd (7.5, 10.5)	3.97 dd (7.0, 11.0)	
Glc-1	5.03 d (7.0)	5.04 d (8.0)	4.96 d (7.5)
Glc-2	4.30 dd (7.0, 9.0)	4.28 dd (8.0, 9.0)	*ca.* 4.21
Glc-3	4.27 dd (9.0, 9.0)	4.30 dd (9.0, 9.0)	*ca.* 4.22
Glc-4	4.17 dd (9.0, 9.0)	4.17 dd (9.0, 9.0)	4.14 dd (9.0, 9.0)
Glc-5	*ca.* 3.90	3.90 ddd (1.5, 5.0, 9.0)	4.00 m
Glc-6a	4.51 dd (2.0, 12.5)	4.51 dd (1.5, 12.0)	4.78 dd (2.0, 11.0)
Glc-6b	4.36 dd (5.5, 12.5)	*ca.* 4.36	4.33 dd (5.5, 11.0)
Rha-1	6.38 d (2.0)	6.38 s	6.34 s
Rha-2	4.82 dd (2.0, 3.0)	4.81 br s	4.79 br s
Rha-3	4.63 dd (3.0, 9.0)	4.64 dd (3.0, 9.0)	4.62 dd (3.0, 9.0)
Rha-4	4.36 dd (9.0, 9.0)	4.36 dd (9.0, 9.0)	*ca.* 4.35
Rha-5	*ca.* 5.00	*ca.* 5.00	*ca.* 4.96
Rha-6	1.78 d (6.0)	1.78 d (6.0)	1.79 d (6.0)
Glc′-1			5.09 d (8.0)
Glc′-2			4.04 dd (8.0, 8.5)
Glc′-3			*ca.* 4.22
Glc′-4			*ca.* 4.22
Glc′-5			*ca.* 3.93
Glc′-6a			4.52 br d (11.5)
Glc′-6b			*ca.* 4.36

δ in ppm from tetramethylsilane (TMS) (coupling constants (J) in Hz are given in parentheses). Assignments are based on ^{1}H–^{1}H COSY, HMQC and HMBC spectra. Glc, glucopyranosyl; Rha, rhamnopyranosyl.

Table 2. ^{13}C-NMR Data for Aglycone Moiety of **1**—**3**, **7**, **8**, **10**, **12** and **14** (in C_5D_5N, 125 MHz)

C	**1**	**2**	**3**	**7**	**8**	**10**	**12**	**14**
1	37.6	37.6	37.6	37.5	37.6	37.6	37.6	37.2
2	30.2	30.2	30.4	30.3	30.3	30.2	30.3	30.1
3	78.1	78.0	78.4	78.2	78.1	78.0	77.9	78.6
4	39.0	39.0	39.2	39.4	39.1	39.0	39.1	38.8
5	140.9	140.9	141.0	141.0	141.0	140.8	141.0	141.0
6	121.8	121.7	121.6	121.7	121.8	121.8	121.8	121.3
7	32.5	32.4	32.4	32.4	32.5	32.4	32.3	31.7
8	32.4	32.3	32.4	32.4	32.4	32.4	32.2	30.8
9	50.3	50.2	50.2	50.3	50.3	50.3	50.4	49.9
10	37.2	37.1	37.2	37.1	37.2	37.2	37.2	37.1
11	21.0	20.9	21.0	21.0	21.0	21.0	21.0	20.9
12	31.8	31.8	31.8	32.1	31.9	31.8	32.0	38.9
13	45.2	45.3	45.1	45.1	45.2	45.1	45.1	43.5
14	53.1	53.0	53.0	53.1	53.1	53.1	53.1	50.5
15	32.1	32.1	32.1	31.8	32.1	32.1	32.5	36.1
16	90.1	90.2	90.1	90.2	90.1	90.1	90.6	210.4
17	90.2	90.1	90.2	90.2	90.2	90.2	90.8	142.6
18	17.2	17.1	17.1	17.2	17.2	17.1	17.3	15.8
19	19.5	19.4	19.5	19.5	19.5	19.4	19.5	19.3
20	44.9	45.3	44.8	44.8	44.8	44.8	43.6	145.6
21	9.7	9.4	9.7	9.7	9.7	9.6	10.3	16.8
22	110.3	110.5	109.9	109.9	109.9	109.8	111.4	205.7
23	31.8	27.4	32.1	32.1	31.9	31.8	36.8	38.0
24	23.6	21.2	28.8	28.8	28.8	28.8	28.0	28.0
25	39.0	36.1	30.5	30.5	30.5	30.4	34.3	33.4
26	64.0	61.5	66.7	66.8	66.8	66.7	75.2	75.1
27	64.4	60.6	17.3	17.3	17.3	17.3	17.4	17.4

δ in ppm from TMS.

Table 3. ^{13}C-NMR Data for Sugar Moiety of **1**—**3**, **7**, **8**, **10**, **12** and **14** (in C_5D_5N)

C	**1**[a]	**2**[a]	**3**[a]	**7**[a]	**8**[a]	**10**[a]	**12**[a]	**14**[a]	Methyl glycoside[b]	
									α	β
Glc-1	100.4	100.4	100.7	102.7	100.4	100.4	100.5	100.4	101.2	105.4
2	79.6	79.6	79.5	75.4	79.7	80.4	79.6	79.6	73.7	74.8
3	77.9	77.9	78.5[c]	78.6[c]	77.9	76.9	78.1[c]	77.9[c]	75.3	78.1
4	71.9	71.9	71.7	71.8	71.9	78.0[c]	71.8	71.9	72.0	71.4
5	78.2	78.2	76.8	78.4[c]	78.2	78.1[c]	78.1[c]	78.2[c]	73.9	78.1
6	62.7	62.7	69.9	62.9	62.8	61.3	62.8	62.7	62.7	62.5
Rha-1	102.0	102.0	102.0		102.1	102.1	102.0	102.0	102.4	102.7
2	72.8	72.8	72.8		72.9	72.8[d]	72.8	72.8	71.9	72.2
3	72.6	72.5	72.5		72.6	72.6[d]	72.5	72.5	72.5	75.4
4	74.2	74.2	74.2		74.2	74.0	74.2	74.2	73.6	73.8
5	69.5	69.5	69.4		69.5	69.5	69.4	69.4	69.4	73.5
6	18.7	18.6	18.6		18.7	18.6	18.6	18.6	18.4	18.5
Glc′-1			105.5							
2			75.2							
3			77.6							
4			71.7							
5			78.4[c]							
6			62.8							
Rha′-1						103.2				
2						73.2				
3						72.8[d]				
4						77.7				
5						68.4				
6						18.8				
Rha″-1						102.2				
2						72.8[d]				
3						72.4[d]				
4						74.1				
5						69.5				
6						18.4				
26-*O*-Glc-1							104.9	104.9		
2							75.2	75.2		
3							78.4[c]	78.4[c]		
4							72.0	71.8		
5							78.6[c]	77.9[c]		
6							62.9	62.9		

δ in ppm from TMS. *a*) at 125 MHz. *b*) Values from ref. 14. *c*, *d*) Assignments in each column may be interchangeable. Glc, glucopyranosyl; Rha, rhamnopyranosyl.

niques similar to those of **1**, were closely comparable to those of **1**, although the resonances of the signals due to the F-ring were slightly different (Tables 2, 3). In addition, the coupling constant values [δ 4.14 (dd, $J=2.0$, 11.0 Hz), 3.94 (br d, $J=11.0$ Hz)] of signals due to H_2-26 indicated **2** to be the 25-epimer of **1**. Thus **2** was concluded as (25*R*)-27-hydroxypenogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside.

Compound **3**, named trikamsteroside B, was obtained as an amorphous powder and analyzed for the molecular formula to be $C_{45}H_{72}O_{18}$ by HR-positive FAB-MS. The ^{1}H- and ^{13}C-NMR spectra of **3** gave signals analogous to those of **8**, with additional signals due to one hexosyl group. Acidic hydrolysis of **3** gave D-glucose and L-rhamnose, which were confirmed by optical rotation using chiral detection in HPLC analyses. Furthermore, the coupling constants (Table 1) of the anomeric and methine proton signals as well as the chemical shifts of ^{13}C-signals[14] (Table 3) due to the sugar moiety indicated that all the monosaccharide units are pyranose form, and that the mode of glycosidic linkages of the two glucosyl groups are β in $^4C_1$ conformation while that of the rhamnosyl group is α in $^1C_4$ conformation. From these findings, it was presumed that **3** is attached by one more glucopyranosyl group to **8**. The ^{13}C-NMR data of **3**, in comparison with those of **8**, exhibited the glycosylation shifts by −1.4 and +7.1 ppm at C-5 and C-6 of the inner glucosyl group (Glc), respectively; in contrast, remaining signals were superimposable on those of **8** (Tables 2, 3). Moreover, the HMBC spectrum indicated a cross-peak between H-1 of the terminal glucosyl group (Glc′) and C-6 of Glc. The structure of **3** was therefore defined as penogenin 3-*O*-β-D-glucopyranosyl-(1→6)-[*O*-α-L-rhamnopyranosyl-(1→2)]-*O*-β-D-glucopyranoside (Fig. 1).

Compound **4**, named trikamsesquiside A, was obtained as an amorphous powder and gave an $[M+Na]^+$ ion peak at *m*/*z* 457 in positive FAB-MS. The molecular formula of **4** was determined as $C_{21}H_{38}O_9$ by HR-positive FAB-MS. The ^{1}H-NMR spectrum (in CD_3OD) of **4** showed signals due to three tertiary methyl groups (δ 1.64, 1.16, 1.14), one olefinic proton [δ 5.27 (br t, $J=6.5$ Hz)], two *exo*-methylene protons [δ 5.10 (s), 4.93 (s)], and one monosaccharide group, which was identified as a β-D-glucosyl group by similar methods to those of **1**. The ^{13}C-NMR spectrum (in CD_3OD) of **4** showed 21 carbon signals including four olefinic carbons (δ 150.5, 136.3, 126.1, 111.2), one oxygenated quaternary carbon (δ 73.5), two oxygenated methine carbons (δ 89.2, 76.6), one oxygenated methylene carbon (δ 66.6), and one β-glucopyranosyl group (δ 105.2, 78.1 (×2), 75.4, 71.6, 62.6). These ^{1}H-

Table 4. ^{1}H-NMR Data for **4** and **16** (500 MHz)

H	**4**[a)]	**4**[b)]	**16**[b)]
1a	3.59 dd (4.0, 11.0)	4.14 dd (4.0, 11.0)	5.31 d (17.5)
1b	3.46 dd (7.5, 11.0)	4.05 dd (7.5, 11.0)	5.10 d (12.5)
2	4.10 dd (4.0, 7.5)	4.69 dd (4.0, 7.5)	6.46 dd (12.5, 17.5)
4a	*ca.* 2.19	*ca.* 2.44	*ca.* 2.26
4b	2.05 ddd (7.5, 7.5, 15.5)	*ca.* 2.30	*ca.* 2.26
5a	*ca.* 2.19	*ca.* 2.34	*ca.* 2.26
5b	*ca.* 2.19	*ca.* 2.34	*ca.* 2.26
6	5.27 br t (6.5)	5.50 t (6.5)	5.45 br t (6.0)
8a	2.36 ddd (4.5, 9.0, 14.0)	2.83 ddd (4.5, 9.5, 14.5)	2.80 ddd (4.5, 9.0, 14.0)
8b	2.13 ddd (7.5, 9.0, 14.0)	*ca.* 2.48	2.47 ddd (8.0, 9.5, 14.0)
9a	1.69 dddd (2.5, 7.5, 9.0, 14.5)	1.87 m	1.87 m
9b	1.58 dddd (4.5, 9.0, 9.0, 14.5)	1.78 dddd (4.5, 9.5, 9.5, 14.0)	1.77 dddd (4.5, 9.5, 9.5, 14.0)
10	3.46 dd (2.5, 9.0)	3.85 dd (1.5, 9.5)	3.79 dd (1.5, 9.5)
12	1.16 s	1.42 s	1.38 s
13	1.14 s	1.35 s	1.33 s
14	1.64 s	1.64 s	1.63 s
15a	5.10 s	5.54 s	5.11 s
15b	4.93 s	5.12 s	5.08 s
Glc-1	4.35 d (7.5)	4.99 d (8.0)	4.92 d (8.0)
Glc-2	3.24 dd (7.5, 9.0)	4.09 dd (8.0, 9.0)	4.04 dd (8.0, 9.0)
Glc-3	3.37 dd (9.0, 9.0)	4.27 dd (9.0, 9.0)	4.25 dd (9.0, 9.0)
Glc-4	*ca.* 3.31	4.23 dd (9.0, 9.0)	4.31 dd (9.0, 9.0)
Glc-5	*ca.* 3.29	*ca.* 4.05	3.99 ddd (2.5, 5.0, 9.0)
Glc-6a	3.85 dd (2.0, 12.0)	4.56 dd (1.5, 11.5)	4.51 dd (2.5, 11.5)
Glc-6b	3.66 dd (5.5, 12.0)	4.34 dd (5.5, 11.5)	4.26 dd (5.0, 11.5)
Glc′-1			5.16 d (8.0)
Glc′-2			4.05 dd (8.0, 9.0)
Glc′-3			4.19 dd (9.0, 9.0)
Glc′-4			4.14 dd (9.0, 9.0)
Glc′-5			3.94 ddd (3.0, 3.0, 9.0)
Glc′-6a			*ca.* 4.48
Glc′-6b			*ca.* 4.48

δ in ppm from TMS (coupling constants (J) in Hz are given in parentheses). Assignments are based on ^{1}H–^{1}H COSY, HMQC and HMBC spectra. *a*) In CD_3OD. *b*) In C_5D_5N. Glc, glucopyranosyl.

Table 5. ^{13}C-NMR Data for **4**, **16**, **22** and **23**

C	**4**[a)]	**4**[b)]	**16**[b)]	**22**[c)]	**23**[c)]
1	66.6	66.8	113.5	111.2	111.3
2	76.6	76.3	139.3	147.2	147.1
3	150.5	151.2	146.4	72.4[e)]	72.5[e)]
4	33.5	33.0	31.7	43.4	43.5
5	27.6	27.1	26.9	23.4	23.5
6	126.1	125.2	124.8	125.8	128.5
7	136.3	[d)]	135.8	135.2	135.2
8	37.0	36.4	36.2	31.2	30.7
9	31.3	31.1	30.9	36.5	36.7
10	89.2	90.1	89.8	90.3	90.7
11	73.5	71.9	71.9	72.0[e)]	73.6[e)]
12	24.5	25.2	25.1	25.2[f)]	24.3[f)]
13	26.6	26.9	26.7	27.0[f)]	26.8[f)]
14	16.0	16.1	16.0	16.1	16.4
15	111.2	110.1	116.2	28.4	28.6
Glc-1	105.2	106.0	105.5	106.1	106.8
Glc-2	75.4	75.5	74.7[e)]	75.5	76.2
Glc-3	78.1	78.4	76.4[f)]	78.6[g)]	78.8[g)]
Glc-4	71.6	71.8	81.0	71.8	71.8
Glc-5	78.1	78.6	76.6[f)]	78.5[g)]	78.3[g)]
Glc-6	62.6	62.7	62.3	62.7	62.9
Glc′-1			104.8		
Glc′-2			74.9[e)]		
Glc′-3			78.1		
Glc′-4			71.4		
Glc′-5			78.3		
Glc′-6			61.8		

δ in ppm from TMS. *a*) In CD_3OD, 125 MHz. *b*) In C_5D_5N, 125 MHz. *c*) Values (in C_5D_5N) from ref. 16. *d*) Signal was oberlapping with that of C_5D_5N. *e*, *f*, *g*) Assignments in each column may be interchangeable. Glc, glucopyranosyl.

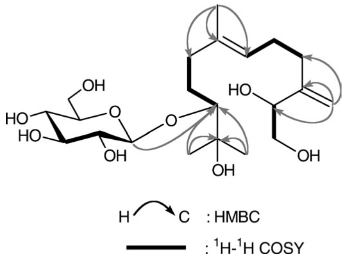

Fig. 2. Connectivities Elucidated by the ^{1}H–^{1}H COSY Spectrum (Bold Lines) and ^{1}H–^{13}C Long-Range Correlations (Arrows) Observed for **4** in the HMBC Spectrum (in CD_3OD, 500 MHz)

and ^{13}C-NMR signals (Tables 4, 5) were examined in detail, and **4** was elucidated to be a farnesane-type sesquiterpenoid glucoside having a planar structure as shown in Fig. 2. In the NOESY spectrum (in C_5D_5N) of **4**, a key NOE correlation was observed between H_3-14 and H_2-5, indicating the geometry of the double bond between C-6 and C-7 to be *E*. The absolute configuration at C-10 of **4** was confirmed by the following evidence. It was reported that the values of glycosylation shift of α-, β-(pro-*S* side), and β'-(pro-*R* side) carbons of secondary alcohols to which β-D-glucopyranose was attached, and the anomeric carbon reflected the absolute configuration of the alcohols.[14,15] Furthermore, the ^{13}C chemical shift differences at C-8, C-11, and C-12 of (10*R*,6*E*)-3,7,11-trimethyl-1,6-dodecadien-3,10,11-triol 10-*O*-glucopyranoside, icariside C_4 (**22**), and its 10-epimer, icariside C_1 (**23**),

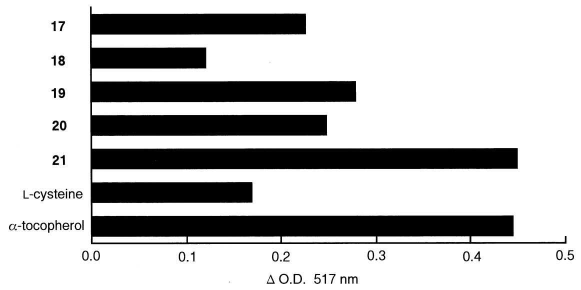

Fig. 3. DPPH Radical-Scavenging Effects of **17**—**21**, L-Cysteine and α-Tocopherol

The final concentration of each tested sample was 0.02 mM. ΔO.D.=O.D. of control at 517 nm (1.1182)$-$O.D. of sample. DPPH; 0.1 mM.

owing to the difference in the configuration at C-10 were observed as in the literature (Table 5),[16] although the assignments at C-8 and C-9 of **22** and **23** in the literature should be interchanged. In addition, the absolute configuration at C-10 of **16** was defined as *R* by synthesizing its aglycone.[9,17] Since the chemical shifts of C-8—C-13 of **4** were quite similar to those of **16** and **22**, the absolute configuration of **4** was considered the same as that of **16** (Table 5). Consequently, **4** was elucidated to be (10*R*,6*E*)-7,11-dimethyl-3-mehylene-6-dodecaene-1,2,10,11-tetraol 10-*O*-β-D-glucopyranoside (Fig. 1).

To the best of our knowledge, **2**, **3**, and **4** are new compounds and the isolation of **1**, **5**—**7**, and **15**—**21** from *T. kamtschaticum* and the isolation of **14** as natural product are described here for the first time.

The scavenging effect of **17**—**21** on the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was examined. All of these compounds except **18** exhibited stronger activity than that of L-cysteine at a concentration of 0.02 mM, and **21** showed the strongest activity among the tested compounds, almost the same as that of α-tocopherol (Fig. 3).

Experimental

All instruments and materials used were the same as those cited in a previous report[18] unless otherwise specified.

Plant Material The underground parts of *T. kamtschaticum* were collected in Hokkaido prefecture, Japan, in May 2003 and identified by Professor Toshihiro Nohara, Faculty of Pharmaceutical Sciences, Kumamoto University.

Extraction and Isolation The cut and air-dried underground parts of *T. kamtschaticum* (279 g) were extracted with MeOH at room temperature and the solvent was removed under reduced pressure to give a syrup (87.1 g). The MeOH extract was subjected to Diaion HP20 (H_2O, MeOH, acetone) to give fraction (fr.) 1—3. A part (25.7 g) of fr. 2 (27.2 g) was chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (20 : 1 : 0, 14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1)] to give fr. 4—12. Fraction 4 (181 mg) and fr. 8 (581 mg) were each successively subjected to Chromatorex ODS [fr. 4 (70% MeOH, MeOH); fr. 8 (40% MeOH, 60% MeOH, 70% MeOH, 80% MeOH, MeOH)] and HPLC [fr. 4 (column, COSMOSIL 5SL-II Nacalai Tesque, Inc., 250 mm×20 mm i.d.; solvent, hexane–acetone (5 : 1)); fr. 8 (column, COSMOSIL 5C18 AR-II, Nacalai Tesque, Inc., 250 mm×20 mm i.d.; solvent, 90% MeOH)] to give **17** (5 mg) from fr. 4 and **7** (4 mg) from fr. 8. Chromatography of fr. 9 (3094 mg) over Chromatorex ODS (60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, MeOH) furnished fr. 13—31. Fraction 14 (527 mg) was chromatographed over Chromatorex ODS (35% MeOH, 40% MeOH, 50% MeOH, MeOH) to afford fr. 32—37. Fraction 13 (159 mg), fr. 18 (182 mg), fr. 21 (171 mg), fr. 34 (126 mg), and fr. 36 (79 mg) were each subjected to HPLC under the similar conditions [fr. 13 (40% MeOH); fr. 18 (60% MeOH); fr. 21 (70% MeOH); fr. 34 (30% MeOH); fr. 36 (55% MeOH)] as fr. 8 to give **19** (14 mg) and **20** (12 mg) from fr. 13, **2** (5 mg) and **1** (33 mg) from fr. 18, **16** (74 mg) from fr. 21, **6** (13 mg) and **5** (11 mg) from fr. 34, and **21** (13 mg), **18** (4 mg), and **4** (6 mg) from fr. 36. Fraction 23 (200 mg) was chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0)] to give **8** (4 mg), **9** (80 mg), and fr. 38—40. Fraction 10 (6586 mg) was subjected to silica gel [$CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0)] to afford fr. 41—49. Chromatography of fr. 42 (16 mg) over Chromatorex ODS (70% MeOH, 80% MeOH, 90% MeOH, MeOH) gave **8** (5 mg). Fraction 44 (37 mg) was subjected to Chromatorex ODS (60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, MeOH) to furnish **1** (6 mg), **9** (4 mg), and fr. 50—52. Similar HPLC (80% MeOH) of fr. 47 (98 mg) as fr. 8 afforded **10** (12 mg). Fraction 48 (4035 mg) was chromatographed over Chromatorex ODS (60% MeOH, 70% MeOH, 80% MeOH, MeOH) to give fr. 53—61. Fraction 54 (719 mg) was subjected to silica gel [$CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0)] to afford fr. 62—66. Fraction 60 (21 mg), fr. 63 (207 mg), fr. 64 (87 mg), and fr. 65 (332 mg) were each subjected to HPLC under similar conditions [fr. 60 (70% MeOH); fr. 63 (55% MeOH); fr. 64 (55% MeOH); fr. 65 (55% MeOH)] as fr. 8 to give **3** (4 mg) from fr. 60, **14** (8 mg), **12** (7 mg), and fr. 67 from fr. 63, **15** (5 mg), **13** (13 mg), and **12** (7 mg) from fr. 64, and **13** (71 mg), **11** (18 mg), and **12** (14 mg) from fr. 65. Chromatography of fr. 67 (50 mg) over silica gel [$CHCl_3$–MeOH–H_2O (10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5)] gave **12** (34 mg).

1: Amorphous powder. $[\alpha]_D^{26}$ $-82.6°$ (c=2.1, MeOH). Positive FAB-MS *m/z*: 777 $[M+Na]^+$. HR positive FAB-MS *m/z*: 777.4120 (Calcd for $C_{39}H_{62}O_{14}Na$: 777.4037). ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Tables 2 and 3.

2: Amorphous powder. $[\alpha]_D^{26}$ $-90.9°$ (c=0.7 MeOH). Positive FAB-MS *m/z*: 777 $[M+Na]^+$, 755 $[M+H]^+$. HR positive FAB-MS *m/z*: 777.4124 (Calcd for $C_{39}H_{62}O_{14}Na$: 777.4037). ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Tables 2 and 3.

3: Amorphous powder. $[\alpha]_D^{31}$ $-104.8°$ (c=0.4, MeOH). Positive FAB-MS *m/z*: 923 $[M+Na]^+$. HR positive FAB-MS *m/z*: 923.4633 (Calcd for $C_{45}H_{72}O_{18}Na$: 923.4617). ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Tables 2 and 3.

4: Amorphous powder. $[\alpha]_D^{14}$ $-1.4°$ (c=0.6, MeOH). Positive FAB-MS *m/z*: 457 $[M+Na]^+$. HR positive FAB-MS *m/z*: 457.2473 (Calcd for $C_{21}H_{38}O_9Na$: 457.2414). ^{1}H-NMR spectral data: see Table 4. ^{13}C-NMR spectral data: see Table 5.

Acidic Hydrolysis of 3 and 4 Compounds **3** (2 mg) and **4** (1 mg) in 2 N HCl (1 ml) were each heated at 95 °C for 1 h. The reaction mixture was neutralized with Amberlite MB-3 then evaporated under reduced pressure to give a residue. The residue was extracted with MeOH and the MeOH extract analyzed by HPLC under the following conditions: column, YMC pack Polyamine II (YMC Co., Ltd., 4.6 mm i.d.×250 mm); solvent, 80% CH_3CN; flow rate, 1.0 ml/min; column temperature, 35 °C; detector, JASCO OR-2090 plus; pump, JASCO PU-2080; and column oven, JASCO CO-2060. The retention time and optical activity of the sample for **3** were identical with those of L-rhamnose [t_R (min) 8.3; optical activity, negative] and D-glucose [t_R (min) 17.8; optical activity, positive], and those for **4** were identical with D-glucose.

Assay of Scavenging Effect on DPPH The method of Uchiyama *et al.*[19] was applied in a slightly modified manner. The EtOH solution (1.00 ml) of each test sample was added to a mixture of 0.1 M acetic acid buffer (pH 5.5, 1.00 ml) and 0.5 mM DPPH EtOH solution (0.50 ml) in a test tube and left to stand at room temperature for 30 min. The absorbance of the resulting solution was measured at 517 nm. α-Tocopherol and L-cysteine were used as standard samples.

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