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Antiviral isoflavonoid sulfate and steroidal glycosides from the fruits of *Solanum torvum*

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Abstract

The C-4 sulfated isoflavonoid, torvanol A (**1**), and the steroidal glycoside, torvoside H (**3**), together with the known glycoside, torvoside A (**2**), were isolated from a MeOH extract of *Solanum torvum* fruits. Upon enzymatic hydrolysis with β-glucosidase, torvoside A (**2**) and torvoside H (**3**) yielded the corresponding acetal derivatives **4** and **5**, respectively. Torvanol A (**1**), torvoside H (**3**) and compound **5** exhibited antiviral activity (herpes simplex virus type 1) with IC_{50} values of 9.6, 23.2 and 17.4 μg/ml, respectively. Compounds **1**–**5** showed no cytotoxicity (at 50 μg/ml) against BC, KB and Vero cell lines.

Keywords: *Solanum torvum*; Solanaceae; Isoflavonoid; Steroidal glycoside; Antiviral compounds; Herpes simplex virus

1. Introduction

Solanum torvum Swartz (Solanaceae) is a small shrub distributed widely in Thailand. Its edible fruits, commonly available in the markets, are used as a vegetable and are regarded as an essential ingredient in Thai cuisine. To date, steroidal glycosides (Mahmood et al., 1985; Agrawal et al., 1989; Cuervo et al., 1991; Yahara et al., 1996; Fayez and Saleh, 1967), and long chain hydrocarbons and steroids (Mahmood et al., 1983) have been previously isolated from *S. torvum*. However, little is known on the biological activities of these isolated compounds. We report herein the isolation, structure elucidation, and biological activities of three polar constituents (**1**–**3**) in the fruits of *S. torvum*. A new isoflavonoid sulfate, named torvanol A (**1**), and a new steroidal glycoside, named torvoside H (**3**), together with a known glycoside, torvoside A (**2**), have been isolated from a MeOH extract of *S. torvum* fruits.

2. Results and discussion

Torvanol A (**1**) was obtained from a MeOH extract of *S. torvum* fruits after sequential purification with gel filtration chromatography on Sephadex LH-20 and preparative (reversed phase C_{18}) HPLC. Although torvanol A (**1**) possesses hydrophobic components (e.g. two aromatic rings and two methyl ethers, it dissolves remarkably well in water. The ^{1}H NMR spectrum (D_2O) of torvanol A (**1**) showed two prominent methyl ether singlets (δ_H 3.75 and 3.84), non-equivalent methylene protons attached to a carbon bearing an oxygen atom (δ_H 4.17 and 4.25), methine protons (δ_H 3.50 and 5.53), two *trans* olefinic protons (δ_H 6.31 and 7.24), and five aromatic protons (δ_H 6.72–7.10). The *J* value of 15.7 Hz of olefinic protons (H-7′ and H-8′) revealed a *trans* geometry of the double bond between C-7′ and C-8′. The ^{13}C NMR spectrum of torvanol A (**1**) revealed 20 signals which were classified by the DEPT technique as nine methine, one methylene, two methyl, and eight quaternary carbons. The ^{1}H–^{1}H COSY spectrum of torvanol A (**1**) revealed a partial structure from H-2 to H-4, and the existence of an ABX system of the aromatic ring A, as well as a *meta* coupling between aromatic protons H-2′ and H-4′. The HMBC spectral data

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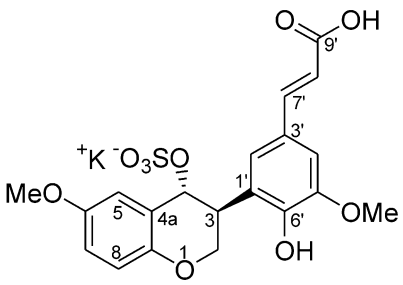

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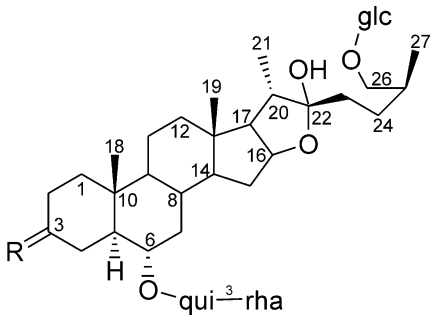
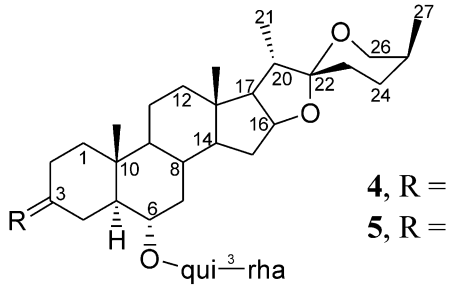


2, R = OH (β-equatorial), H
3, R = O



4, R = OH (β-equatorial), H
5, R = O

(optimized for $^{n}J_{HC}=8.0$ Hz) of torvanol (**1**) showed correlations of H-2 to C-4 and C-1′; H-3 to C-4a and C-1′; H-4 to C-2 and C-5; H-5 to C-4, C-7 and C-8a; H-7 to C-5 and C-8a; H-8 to C-4a and C-6; both H-2′ and H-4′ to C-6′ and C-7′; H-7′ to C-2′, C-4′ and C-9′; and H-8′ to C-3′. These ^{1}H–^{1}H COSY and HMBC spectral data readily revealed that torvanol A (**1**) possesses an isoflavonoid skeleton. Both methoxy groups were assigned by analyses of the HMBC and NOESY spectral data. The HMBC spectrum of torvanol A (**1**) showed correlation of 6-OMe protons to C-6, and 5′-OMe protons to C-5′, while the NOESY spectrum of (**1**) revealed cross peaks between 6-OMe protons and H-5, as well as between 5′-OMe protons and H-4′. The chemical shifts at C-4 (δ_H 5.53 and δ_C 88.3) suggested the presence of an ester in torvanol A (**1**), however, there were no further carbon resonances on the ^{13}C NMR of torvanol A (**1**), implying the existence of an inorganic ester in **1**. The accurate mass of *m*/*z* 451.0677 [$(M–H)^-$, Δ−2.2 mmu] obtained from the ESITOF mass spectrum (negative ion mode) of torvanol A (**1**) revealed a molecular formula of **1** as $C_{20}H_{20}O_{10}S$, suggesting that **1** possessed a sulfate ester. The IR spectrum of **1** indicated the presence of hydroxyl groups (ν_{max} 3448 cm^{-1}), conjugated carbonyl (ν_{max} 1638 cm^{-1}), and sulfate ester (ν_{max} 1256 cm^{-1}). The presence of a sulfate ester group was also indicated by the EIMS spectrum of torvanol A (**1**), showing the prominent peak of the fragment ion at *m*/*z* 355 [M-97], due to the loss of $[HSO_4]^-$. The solubility of torvanol A (**1**) in water suggested that **1** existed in the form of a sulfate salt. Analysis by an atomic absorption spectrophotometer revealed that **1** was a potassium salt, showing the stoichiometric ratio ca. 1:1 (torvanol A (**1**) and K). On the basis of these spectral data, the chemical structure of torvanol A (**1**) was therefore secured. The assignments of protons and carbons of torvanol A (**1**) are shown in Table 1.

The $^{3}J_{3,4}$ value of 6.4 Hz suggested a pseudo axial orientation of H-4, and the respective $J_{H\text{-}2ax,H\text{-}3}$ and $J_{H\text{-}2eq,H\text{-}3}$ of 7.0 and 4.7 Hz revealed that H-3 was also pseudo axial. The NOESY spectrum of torvanol A (**1**) showed correlations between H-4 and H-2ax, and H-3 and H-2eq, supporting the proposed relative stereochemistry.

The ^{1}H NMR spectrum (pyridine-d_5) of torvoside H (**3**) ($[\alpha]_D^{29}$ −58.15°, *c* 0.114 in MeOH) was similar to that of the known glycoside, torvoside A (**2**) ($[\alpha]_D^{29}$ −50.16°, *c* 0.123 in MeOH). The ^{13}C NMR spectrum of torvoside H (**3**) and that of **2** were also similar, except that the hydroxy bearing carbon signal at δ_C ca. 71.0 (C-3) in **2** was replaced by a signal at δ_C 210.7 in **3**, characteristic of a ketone functionality. The ketone moiety was also confirmed by an absorption peak at 1702 cm^{-1} in the IR spectrum of **3**. The ESITOF mass spectral data (negative ion mode) revealed quasi-molecular ions at *m*/*z* 901 $[M–H]^-$ for torvoside H (**3**) and 903 $[M–H]^-$ for torvoside A (**2**), implying the hydroxyl group at C-3 in **2** was a ketone in **3**. On enzymatic hydrolysis with β-glucosidase, torvoside H (**3**) gave glucose and compound **5**, suggesting that **3** possessed a glucose unit at C-26, which is the same as that of torvoside A (**2**) (Yahara et al., 1996). The ESITOF mass spectrum (negative ion mode) established the molecular formula of compound **5** as $C_{39}H_{62}O_{12}$, showing an accurate mass at *m*/*z* 721.4155

Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data (D_2O) of torvanol A (**1**)

C	Torvanol A (**1**)	
	δ_C, multiplicity[a]	δ_H, multiplicity, J in Hz
1	–	–
2	69.3, *t*	4.17, *dd*, 10.1, 7.0 (H-2ax) 4.25, *dd*, 10.1, 4.7 (H-2eq)
3	50.4, *d*	3.50, *m*
4	88.3, *d*	5.53, *d*, 6.4
4a	132.8, *s*	–
5	110.2, *d*	6.89, *d*, 1.0
6	148, *s*	–
7	118.9, *d*	6.76, *dd*, 8.1, 1.0
8	115.9, *d*	6.80, *d*, 8.1
8a	145.6, *s*	–
1′	127.6, *s*	–
2′	112.5, *d*	7.02, *br s*
3′	130.2, *s*	–
4′	117.4, *d*	7.08, *br s*
5′	144.0, *s*	–
6′	148.7, *s*	–
7′	141.2, *d*	7.24, *d*, 15.7
8′	122.5, *d*	6.31, *d*, 15.7
9′	176.2, *s*	–
6-OMe	56.2, *q*	3.75, *s*
5′-OMe	56.4, *q*	3.84, *s*

[a] Multiplicity was determined by analyses of the DEPT spectra.

[(M–H)$^-$, $\Delta-0.8$ mmu]. The ^{1}H and ^{13}C NMR spectral data of **5** were less complex than that of torvoside H (**3**), particularly in the sugar's resonances, which enabled the assignment of protons and carbons in **5**. Analyses of DEPT, ^{1}H–^{1}H COSY, HMQC and HMBC spectral data led to a complete assignment of both protons and carbons in the molecule (Table 2). Important ^{1}H–^{13}C long-ranged correlations on the HMBC spectrum of compound **5** were as follows: H-2 and H-4 to C-3; H-4 to C-6; H-18 to C-1 and C-5; H-19 to C-12, C-14 and C-17; H-16 to C-20; H-21 to C-17 and C-22; H-24 to C-22; and H-26 to C-22, C-24 and C-27. The HMBC spectrum of **5** also demonstrated the correlation of the anomeric proton (H-qui1) of quinovose to C-6 and that of the anomeric proton (H-rha1) of rhamnose to C-qui3. These HMBC correlations provided useful information concerning connectivities between quinovose and the steroidal aglycone (linkage at C-6), as well as that between quinovose and rhamnose (1→3 linkage). The downfield shifts of H-qui3 (at δ_H 4.21) and C-qui3 (at δ_C 83.7) in the ^{1}H and ^{13}C NMR spectra of **5** also indicated the connectivity (1→3) of the two sugars. As for the relative configuration of the sugars in **5**, the $J_{H\text{-}qui1,H\text{-}qui2}$ (7.8 Hz), $J_{H\text{-}qui2,H\text{-}qui3}$ (9.0 Hz) and $J_{H\text{-}qui3,H\text{-}qui4}$ (9.0 Hz), revealed axial orientations of H-qui1, H-qui2, H-qui3 and H-qui4, respectively, while those of $J_{H\text{-}rha2,H\text{-}rha3}$ (3.2 Hz), $J_{H\text{-}rha3,H\text{-}rha4}$ (9.3 Hz) and $J_{H\text{-}rha4,H\text{-}rha5}$ (9.3 Hz) readily indicated the respective configurations of H-2, H-3, H-4 and H-5 as equatorial, axial, axial and axial. Finally, the presence of quinovose and rhamnose (1→3 linkage) in **5** was confirmed by comparison of the ^{13}C signals with those of published values (Yahara et al., 1996). Torvoside H (**3**) exhibited a negative optical rotation similar to that of torvoside A (**2**), it is therefore not unreasonable to assume that the absolute configurations of all sugars in **3** are the same as those in **2**. On the basis of these spectral data, compound **5** is (25*S*)-6α-hydroxy-5α-spirostan-3-one 6-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-quinovopyranoside], and torvoside H (**3**) is therefore (25*S*)-26-*O*-(β-D-glucopyranosyl)-6α,26-dihydroxy-5α-spirostan-3-one 6-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-quinovopyranoside].

Torvanol A (**1**), torvoside H (**3**) and compound **5** exhibited antiviral activity (herpes simplex virus type 1) with the IC_{50} values of 9.6, 23.2 and 17.4 μg/ml, respectively. Compounds **1**–**5** were inactive (at 50 μg/ml) against BC, KB and Vero cell lines. Recently, antiviral activity (herpes virus) of *Solanum* steroidal glycosides has been demonstrated (Ikeda et al., 2000), while Chah et al. (2000) reported an antimicrobial activity of the methanolic extract of *S. torvum* fruits.

3. Experimental

3.1. General

The ^{1}H, ^{13}C, DEPTs, ^{1}H–^{1}H COSY, NOESY, HMQC (optimized for $^1J_{HC}=145$ Hz) and HMBC (optimized for $^nJ_{HC}=8.0$ Hz as well as 4.0 Hz) spectra were recorded on a Bruker DRX 400, operating at 400.1 MHz for proton and 100.6 MHz for carbon. The ESITOF mass spectra were obtained from a Micromass LCT mass spectrometer, while the IR spectra were measured on a Perkin-Elmer 2000 spectrometer. An HPLC pump (Waters 600) was equipped with a UV-photodiode array detector (Waters 996). The metal composition in torvanol A (**1**) was analyzed with a Perkin Elmer (model 3100) flame atomic absorption spectrometer.

3.2. Plant specimen, extraction and isolation

Fresh fruits (2.5 kg) of *S. torvum* Swartz were bought from Pak Klong Talad Market, Bangkok, Thailand. Crushed fruits were macerated in MeOH (6 l) for 2 days at room temperature. The extract was evaporated, and dissolved in 70% aqueous MeOH, which was sequentially extracted with hexane and EtOAc (equal volume, three times). Aqueous MeOH, hexane, and EtOAc layers were evaporated to dryness, to give respective yields of 15.3, 10.0, and 5.5 g. A crude extract of the aqueous MeOH layer was applied to a Sephadex LH-20 column (eluted with MeOH). Twenty fractions (ca. 100 ml) were collected and evaporated to dryness in vacuo. Fractions 15–20 containing torvanol A (**1**) were combined, and

Table 2
^1H (400 MHz) and ^{13}C (100 MHz) NMR spectral data (pyridine-d_5) of torvoside H (**3**) and its derivative (**5**)

C	Torvoside H (**3**)		Compound **5**	
	δ_C, multiplicity[a]	δ_H, multiplicity	δ_C, multiplicity[a]	δ_H, multiplicity
1	38.7, *t*	1.52, *m*; 1.76, *m*	38.7, *t*	1.15, *m*; 1.77, *m*
2	38.1, *t*	2.25, *m*; 2.38, *m*	38.1, *t*	2.26, *m*; 2.38, *m*
3	210.7, *s*	–	210.7, *s*	–
4	39.8, *t*	2.36, *m*; 2.46, *m*	39.9, *t*	2.38, *m*; 3.49, *br d*, 14.5
5	52.4, *d*	1.55, *m*	52.4, *d*	1.57, *m*
6	79.9, *d*	3.68, *m*	80.0, *d*	3.64, *m*
7	41.0, *t*	1.10, *m*; 2.41, *m*	41.0, *t*	1.13, *m*; 2.49, *m*
8	34.5, *d*	1.85, *m*	34.0, *d*	1.63, *m*
9	53.2, *d*	0.57, *m*	53.2, *d*	0.57, *ddd*, 10.8, 10.8, 3.6
10	36.7, *s*	–	36.7, *s*	–
11	21.3, *t*	1.23, *m*; 1.39, *m*	21.3, *t*	1.26, *m*; 1.41, *m*
12	39.7, *t*	0.98, *m*; 1.56, *m*	39.9, *t*	1.03, *m*; 1.64, *m*
13	41.1, *s*	–	40.7, *s*	–
14	56.0, *d*	1.00, *m*	56.1, *d*	1.05, *m*
15	32.1, *t*	1.36, *m*; 1.98, *m*	32.1, *t*	1.44, *m*; 2.02, *m*
16	81.2, *d*	4.40, *m*	81.1, *d*	4.45, *ddd*, 7.2, 7.2, 7.2
17	64.2, *d*	1.70, *m*	62.7, *d*	1.73, *m*
18	12.6, *q*	0.95, *s*	12.6, *q*	0.95, *s*
19	16.5, *q*	0.79, *s*	16.3, *q*	0.82, *s*
20	40.5, *d*	2.20, *m*	42.5, *d*	1.86, *m*
21	16.3, *q*	1.14, *d*, 6.6	14.9, *q*	1.12, *m*
22	112.6, *s*	–	109.7, *s*	–
23	30.9, *t*	1.82, *m*; 1.91, *m*	26.4, *t*	1.42, *m*; 1.88, *m*
24	28.2, *t*	1.40, *m*; 1.76, *m*	26.2, *t*	1.37, *m*; 2.13, *m*
25	33.9, *d*	1.54, *m*	27.5, *d*	1.56, *m*
26	75.0, *t*	3.50; 4.06	65.1, *t*	3.33, *br d*, 10.8; 4.01, *dd*, 11.0, 2.1
27	17.6, *q*	1.03, *d*, 6.6	16.6, *q*	1.05, *d*, 6.9
glc-1	105.1, *d*	4.84, *d*, 7.6	–	–
glc-2	75.2, *d*	4.06	–	–
glc-3	78.6, *d*	4.22	–	–
glc-4	71.7, *d*	4.25	–	–
glc-5	78.5, *d*	3.96	–	–
glc-6	62.8, *t*	4.34; 4.55	–	–
qui-1	105.6, *d*	4.67, *d*, 7.8	105.6, *d*	4.66, *d*, 7.8
qui-2	75.9, *d*	3.96	75.9, *d*	3.97
qui-3	83.7, *d*	4.20	83.7, *d*	4.21, *dd*, 9.0, 9.0
qui-4	75.1, *d*	4.00	75.2, *d*	3.58
qui-5	72.7, *d*	3.72	72.7, *d*	3.71
qui-6	18.8, *q*	1.60, *d*, 6.1	18.8, *q*	1.61, *d*, 6.0
rha-1	103.3, *d*	6.23, *br s*	103.3, *d*	6.22, *br s*
rha-2	72.5, *d*	4.81	72.5, *d*	4.81, *br s*
rha-3	72.7, *d*	4.56	72.8, *d*	4.58, *dd*, 9.3, 3.2
rha-4	74.1, *d*	4.32, *dd*, 9.4, 9.4	74.1, *d*	4.33, *dd*, 9.3, 9.3
rha-5	70.0, *d*	4.99	70.0, *d*	4.98
rha-6	18.6, *q*	1.58, *d*, 6.1	18.7, *q*	1.67, *d*, 6.1

[a] Multiplicity was determined by analyses of the DEPT spectra.

subsequently purified by preparative HPLC (C_{18} reversed phase column, Prep Nova Pak, Waters) using H_2O:MeOH (90:10, v/v) as eluent, yielding torvanol A (**1**) (25 mg). Fractions 3–6 obtaining from Sephadex LH-20 column were further purified by MPLC (C_{18} reversed phase column) using H_2O:MeOH (60:40, v/v) as eluent, to yield torvoside A (**2**) (2.1 g) and torvoside H (**3**) (0.2 g).

3.3. Bioassay

The cytotoxic assay employed the colorimetric method (Skehan et al., 1990). Ellipticine, the reference substance, exhibited activity toward BC and KB cell lines, both with the IC_{50} of 0.3 μg/ml. Antiviral activity was also assessed employing the colorimetric method (Skehan et al., 1990). Herpes simplex virus type 1 (HSV-1)

was maintained in the Vero cell line (kidney fibroblast of an African green monkey), which was cultured in Eagle's minimum essential medium (MEM) with addition of heat-inactivated fetal bovine serum (FBS) (10%) and antibiotics. The test samples were put into wells of a microtiter plate at the final concentrations ranging from 20 to 50 μg/ml. The viral HSV-1 (30 PFU) was added into the 96-well plate, followed by plating of Vero cells (1×10^5 cells/ml); the final volume was 200 μl. After incubation at 37 °C for 72 h, under 5% of CO_2 atmosphere, cells were fixed and stained, and optical density was measured at 510 nm. Under our screening conditions, the reference compound, Acyclovir, typically exhibited the antiviral HSV-1 with the IC_{50} of 2-5 μg/ml.

3.4. Torvanol A (*1*)

Amorphous powder: mp > 300 °C $[\alpha]_D^{29}$ −50° (*c* 0.280, H_2O); IR ν_{max} cm^{-1}: 3448, 2362, 1638, 1610, 1545, 1499, 1389, 1330, 1256, 1145, 1065, 985, 851, 822, 761; UV (MeOH) λ_{max} nm: 203, 234, 283; ESITOF MS (negative ion mode): *m/z* 451.0677, calc. for $C_{20}H_{19}O_{10}S$ $(M–H)^-$, free acid form, 451.0699; for ^1H and ^{13}C NMR spectra (Table 1).

3.5. Torvoside H (*3*) and its derivative (*5*)

Torvoside H (**3**) was obtained as an amorphous powder: mp 170–172 °C $[\alpha]_D^{29}$ −58.15° (*c* 0.114, MeOH); IR ν_{max} cm^{-1}: 3422, 2932, 1702, 1655, 1648, 1458, 1420, 1381, 1074; ESITOF MS (negative ion mode): *m/z* 901.465, calc. for $C_{45}H_{73}O_{18}$ $(M–H)^-$, 901.479; for ^1H and ^{13}C NMR spectra (Table 2). Torvoside H (**3**) (70 mg) was incubated with commercial almond β-glucosidase (7 mg) in H_2O (12.5 ml) at 37 °C for 24 h. After evaporation of the solvent, the reaction mixture was applied to a silica gel column, eluted with $CHCl_3$:MeOH (3:1), to give β-D-glucose and a derivative **5** (mp 180–183 °C; $[\alpha]_D^{29}$ −63.72°, *c* 0.113 in MeOH), ESITOF MS (negative ion mode): *m/z* 721.4155, calc. for $C_{39}H_{61}O_{12}$ $(M-H)^-$, 721.4163; ^1H and ^{13}C NMR (Table 2).

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