Cytotoxic Phenylpropanoid Glycosides from the Stems of *Smilax china*

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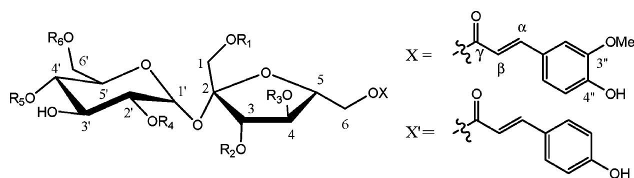
Bioassay-guided fractionation of an ethanol extract of *Smilax china* led to the isolation of nine phenylpropanoids including six new compounds, smilasides A−F (**1**-**6**), and three known phenylpropanoids, smiglaside E, heloniosides B, and 2′,6′-diacetyl-3,6-diferuloylsucrose. Structural elucidation of isolates **1**−**6** was based on spectroscopic data analysis. These new phenylpropanoids were evaluated against several human tumor cell lines.

Smilax china (Smilacaceae) is a perennial herbaceous plant that is distributed throughout southern Asia.[1] The stems of *S. china* have been used in traditional medicine to treat syphilis, gout, and rheumatism in mainland China and Taiwan.[2] Previous investigations have demonstrated that crude extracts of *S. china* have antimutagenic[3] and antioxidant[4] activities, as well as a therapeutic efficacy as an adjuvant in arthritis.[5] While several steroidal saponins have been isolated from *S. china*,[6] there are no reports on the investigation of other potential antitumor constituents of this plant. As part of a search for bioactive agents from the terrestrial plants of Taiwan, we found that an EtOH extract of *S. china* exhibited cytotoxicity against the KB, Hela, and DLD-1 cell lines. Bioassay-directed fractionation of this extract led to the isolation of six new phenylpropanoids, namely, smilasides A−F (**1**−**6**), along with the known smiglaside E, helonioside B, and 2′,6′-diacetyl-3,6-diferuloylsucrose. We report herein on the isolation and structural elucidation of **1**−**6** using spectroscopic data analysis, including 1D and 2D NMR techniques (^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY). Biological evaluation of the newly isolated phenylpropanoids (**1**−**6**) against a panel of cancer cell lines is also described.



	R1	R2	R3	R4	R5	R6
1	H	X	H	H	$COCH_3$	$COCH_3$
2	H	X	H	$COCH_3$	H	H
3	X′	X	H	H	H	H
4	X′	X	$COCH_3$	H	H	H
5	X′	X	H	H	H	$COCH_3$
6	X′	X′	H	$COCH_3$	H	$COCH_3$

Results and Discussion

The EtOH extract of *S. china* was partitioned successively with *n*-hexane, dichloromethane, and *n*-butanol. The extracts obtained were subjected to a series of column chromatographic and/or HPLC steps, resulting in the isolation of six new compounds (**1**−**6**) and three known compounds.

The HRFABMS of compound **1** suggested the elemental formula $C_{36}H_{42}O_{19}$ from a quasi-molecular ion at *m/z* 777.2238 [M − H]$^-$. The IR and UV spectra displayed absorption bands for the hydroxyl and α,*β*-unsaturated aromatic ester groups. In the ^{1}H NMR spectrum (Table 1), signals for seven oxygenated methines (δ_H 5.44, 4.47, 4.19, 5.51, 3.50, 3.70, 4.71), three oxygenated methylenes (δ_H 3.62 and 3.68; 4.45 and 4.55; 4.23 and 4.07), four olefinic protons [δ_H 6.42, 7.70, (d, J = 16.0 Hz); 6.40, 7.65 (d, J = 16.0 Hz)], and two benzyl moieties with AMX coupling patterns [δ_H 6.82 (d, J = 8.4 Hz), 7.13 (dd, J = 8.4, 2.0 Hz), 7.26 (d, J = 2.0 Hz); δ_H 6.81 (d, J = 8.4 Hz), 7.08 (dd, J = 8.4, 2.0 Hz), 7.18 (d, J = 2.0 Hz)] were observed. A characteristic anomeric signal appeared at δ_H 5.51 with a smaller coupling constant (H-1′, d, J = 4.0 Hz), and since the ^{13}C NMR spectrum showed 12 oxygenated carbon signals including those for two anomeric carbons, this suggested that **1** possesses a disaccharide moiety. Alkaline hydrolysis of **1** gave sucrose [α-D-Glc-(1→2)-*β*-D-Fru] as the component sugar, which was confirmed by comparing the HPLC, NMR, and optical rotation data with those of an authentic sample.[7] Moreover, the presence of two pairs of *trans* olefinic protons, along with two methoxy groups at the aromatic moieties as determined from the HMBC spectrum, suggested the presence of two *trans*-ferulic acid units in **1**. On further inspection of the HMBC spectrum, not only the respective ferulic acid at C-3 and C-6 in fructose (Fru) but also the acetyl methyl group at C-4′ and C-6′ of glucose (Glc) could be assigned unambiguously, due to the correlations observed between H-3, H-6, H-4′, and H-6′ and the corresponding carbonyl carbons of ferulate and acetate. Thus, the structure of **1** was characterized as 3,6-diferuloyl-4′,6′-diacetyl sucrose, and this compound was named smilaside A.

Smilaside B (**2**) showed a quasi-molecular ion at *m/z* 735.2128 [M − H]$^-$ by HRFABMS, which is consistent with the molecular formula $C_{34}H_{40}O_{18}$. The ^{1}H and ^{13}C NMR spectra suggested that **2** possesses a structure similar to **1**, containing glucose (Glc) and fructose (Fru) units, and two ferulic acid moieties at C-3 and C-6 of the fructose unit, respectively, but only one acetate group was present in **2**. In the ^{1}H NMR spectrum of **2**, the signal for H-2′ in the Glc unit was shifted to lower field (δ_H 4.58), compared with those of the other oxygenated protons. The HMBC experiment of **2** displayed the correlations between the carbonyl

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Table 1. ^1H NMR Spectroscopic Data (δ in $CDCl_3$) for **1**–**6**[a,b]

H	**1**	**2**	**3**	**4**	**5**	**6**
1	3.68 (d, 12.0)	3.63 (d, 12.0)	4.37 (m)	4.38 (d, 12.0)	4.36 (d, 12.0)	4.29 (d, 12.0)
	3.62 (d, 12.0)	3.48 (d, 12.0)	4.25 (m)	4.28 (d, 12.0)	4.31 (d, 12.0)	4.17 (d, 21.0)
3	5.44 (d, 6.8)	5.54 (d, 8.0)	5.64 (d, 8.5)	5.78 (d, 7.6)	5.62 (d, 8.4)	5.52 (d, 8.0)
4	4.47 (t, 6.8)	4.43 (t, 6.8)	4.54 (m)	5.71 (d, 7.6)	4.61 (d, 8.8)	4.56 (t, 8.0)
5	4.19 (m)	4.12 (m)	4.26 (m)	4.40 (m)	4.20 (m)	4.15 (m)
6	4.45 (dd, 12.0, 6.8)	4.47 (2H, m)[c]	4.58 (2H, m)[c]	4.54 (dd, 12.0, 1.6)	4.57 (2H, m)[c]	4.50 (2H, m)[c]
	4.55 (dd, 12.0, 4.0)			4.56 (m)		
OAc-4				2.09 (s)		
1′	5.51 (d, 4.0)	5.61 (d, 4.0)	5.54 (d, 4.0)	5.57 (d, 3.7)	5.56 (d, 4.0)	5.66 (d, 4.0)
2′	3.50 (dd, 10.0, 4.0)	4.58 (dd, 10.0, 5.0)	3.46 (m)	3.43 (m)	3.44 (dd, 10.0, 4.0)	4.62 (dd, 10.0, 3.6)
3′	3.70 (t, 10.0)	3.83 (t, 10.0)	3.70 (t, 10.0)	3.70 (t, 10.0)	3.64 (t, 9.6)	3.84 (t, 10.0)
4′	4.71 (t, 10.0)	3.44 (t, 10.0)	3.45 (m)	3.46 (t, 9.6)	3.27 (t, 10.0)	3.34 (t, 10.0)
5′	4.27 (ddd, 10.0, 6.0, 2.0)	3.94 (m)	4.02 (m)	4.06 (m)	4.25 (m)	4.14 (m)
6′	4.23 (dd, 12.0, 2.0)	3.88 (m)	3.94 (m)	3.94 (dd, 12.0, 2.4)	4.65 (m)	4.51 (m)
	4.07 (dd, 12.0, 6.0)	3.76 (dd, 12.0, 6.0)	3.85 (m)	3.83 (m)	4.15 (m)	4.15 (m)
OAc-2′		2.07 (s)				2.09 (s)
OAc-4′	1.78 (s)					
OAc-6′	2.03 (s)				2.10 (s)	2.06 (s)
X′-1						
α″			7.63 (d, 16.0)	7.65 (d, 16.0)	7.65 (d, 16.0)	7.64 (d, 16.0)
β″			6.32 (d, 16.0)	6.35 (d, 16.0)	6.35 (d, 16.0)	6.34 (d, 16.0)
2″			7.37 (d, 8.5)	7.41 (d, 8.4)	7.40 (d, 8.4)	7.42 (d, 8.4)
3″			6.74 (d, 8.5)	6.73 (d, 8.4)	6.76 (d, 8.4)	6.74 (d, 8.4)
5″			6.74 (d, 8.5)	6.73 (d, 8.4)	6.76 (d, 8.4)	6.74 (d, 8.4)
6″			7.37 (d, 8.5)	7.41 (d, 8.4)	7.40 (d, 8.4)	7.42 (d, 8.5)
X or X′-3						
α‴	7.70 (d, 16.0)	7.70 (d, 16.0)	7.72 (d, 16.0)	7.71 (d, 16.0)	7.72 (d, 16.0)	7.72 (d, 16.0)
β‴	6.42 (d, 16.0)	6.47 (d, 16.0)	6.45 (d, 16.0)	6.42 (d, 16.0)	6.42 (d, 16.0)	6.45 (d, 16.0)
2‴	7.26 (d, 2.0)	7.25 (d, 1.6)	7.16 (s)	7.21 (d, 2.0)	7.22 (d, 2.0)	7.50 (d, 8.4)
3‴						6.81 (d, 8.4)
5‴	6.82 (d, 8.4)	6.81 (d, 8.0)	6.81 (d, 8.5)	6.82 (d, 8.0)	6.82 (d, 8.4)	6.81 (d, 8.4)
6‴	7.13 (dd, 8.4, 2.0)	7.12 (dd, 8.4, 2.0)	7.10 (d, 8.5)	7.11 (dd, 8.0, 2.0)	7.11 (dd, 8.4, 2.0)	7.50 (d, 8.4)
OMe	3.90 (s)	3.90 (s)	3.86 (s)	3.90 (s)	3.89 (s)	
X-6						
α⁗	7.65 (d, 16.0)	7.66 (d, 16.0)	7.64 (d, 16.0)	7.65 (d, 16.0)	7.66 (d, 16.0)	7.64 (d, 16.0)
β⁗	6.40 (d, 16.0)	6.40 (d, 16.0)	6.39 (d, 16.0)	6.41 (d, 16.0)	6.46 (d, 16.0)	6.41 (d, 16.0)
2⁗	7.18 (d, 2.0)	7.20 (d, 2.0)	7.14 (s)	7.21 (d, 2.0)	7.19 (d, 2.0)	7.20 (s)
5⁗	6.81 (d, 8.4)	6.81 (d, 8.4)	6.81 (d, 8.5)	6.81 (d, 8.0)	6.81 (d, 8.4)	6.80 (dd, 8.4, 1.6)
6⁗	7.08 (dd, 8.4, 2.0)	7.10 (dd, 8.4, 2.0)	7.05 (d, 8.5)	7.09 (dd, 8.0, 2.0)	7.09 (dd, 8.4, 2.0)	7.07 (d, 8.4)
OMe	3.89 (s)	3.89 (s)	3.86 (s)	3.88 (s)	3.89 (s)	3.87 (s)

[a] Assignments were confirmed by ^1H–^1H COSY, TOCSY, HMBC, and HMQC. [b] Compounds **1**, **2**, and **4**–**6** were measured at 400 MHz and **3** at 500 MHz. [c] Overlapped signal.

carbon at δ_C 172.5 and protons at δ_H 4.58 (H-2′ of Glc), confirming that the acetate group was located at C-2′ of Glc. Based on this evidence, **2** (smilaside B) was determined as 3,6-diferuloyl-2′-acetyl sucrose.

Smilaside C (**3**) revealed a molecular formula of $C_{41}H_{44}O_{19}$ from a quasi-molecular ion at *m/z* 839.2406 $[M - H]^-$ by HRFABMS. The IR spectrum of **3** showed strong absorption bands at 3376 (OH) and 1602 (aromatic) cm^{-1}, which, together with the signals in the ^1H and ^{13}C NMR spectra, suggested that **3** might have a structure similar to **2**, except for the absence of an acetate group and the appearance of an additional benzyl moiety and two olefinic protons in **3**. Moreover, the signals for *trans*-olefinic protons [(δ_H 6.32, 7.63 (d, $J = 16.0$ Hz)] and a pair of A_2B_2 pattern protons [(δ_H 7.37 for 2″, 6″ and 6.74 for 3″, 5″ (d, $J = 8.5$ Hz)] observed in the ^1H NMR spectrum revealed that **3** possesses a *trans*-coumaric acid unit. The HMBC spectrum of **3** provided further evidence of a coumaric acid substituent at C-1 of the sucrose. Thus, the structure of **3** (smilaside C) was established as 1-*p*-coumaroyl-3,6-diferuloyl sucrose.

Smilaside D (**4**) showed a quasi-molecular ion at *m/z* 881.2515 $[M - H]^-$ by HRFABMS, which corresponds to the molecular formula $C_{43}H_{46}O_{20}$. The IR and ^1H and ^{13}C NMR spectra of **4** suggested two ferulic acid units, one coumaric acid unit, one glucose, and one fructose, in a manner similar to **3**, but with the presence of an additional acetate group in **4**. In the HMBC spectrum of **4**, the position of the acetate group at C-3 in fructose was deduced, along with the locations of the ferulic and *p*-coumaric acids units, from the long-range correlations between H-1, H-3, and H-6 and the carbonyl carbons of these acids. When this evidence was taken together, the structure of **4** (smilaside D) could be assigned as 1-*p*-coumaroyl-3,6-diferuloyl-4-acetyl sucrose.

By HRFABMS {*m/z* 881.2496 $[M - H]^-$}, smilaside E (**5**) was accorded the molecular formula $C_{43}H_{46}O_{20}$. Moreover, from the IR and ^1H and ^{13}C NMR spectroscopic data similar to those of **4**, it was revealed that these two compounds have related structures. On comparing the ^1H NMR spectra of **4** and **5**, H-4 in **5** was shifted to higher field (δ_H 5.71 for **4**; δ_H 4.61 for **5**), whereas H-6′ was shifted to lower field (δ_H 3.83, 3.94 for **4**; δ_H 4.15, 4.65 for **5**), suggesting that **5** possesses an acetate group at C-6′ of the glucose unit. The assignment of the acetate group at C-6′ was also confirmed by the HMBC spectrum, from a correlation between H-6′ and the carbonyl carbon of acetate group. Accordingly, **5** (smilaside E) was determined as 1-*p*-coumaroyl-3,6-diferuloyl-6′-acetyl sucrose.

Smilaside F (**6**) was obtained as an amorphous light yellowish glass, and its molecular formula was determined to be $C_{44}H_{46}O_{20}$ {*m/z* 893.2491$[M - H]^-$} by HRFABMS. The IR and ^1H and ^{13}C NMR spectra indicated that **6** has a sucrose unit and three glycosidic phenylpropanoid moieties as in isolates **3**–**5**. When the ^1H NMR spectrum of **6** and **5** were compared, the loss of a methoxyl group and the increase of an acetate group in **6** were inferred. This

evidence suggested that **6** possesses one ferulic acid, two coumaric acids, and two acetate units, instead of two ferulic acids, one coumaric acid, and one acetate unit in **5**. The locations of these phenylpropanoids and two acetate groups in **6** were assigned from the HMBC NMR spectrum. Thus, two coumaric acid units were assigned at C-1 and C-3, and a ferulic acid was assigned at C-6 of Fru. Moreover, two acetate groups were assigned unambiguously at C-2′ and C-6′, respectively, due to the long-range correlations between H-2′ and H-6′ and the carbonyl carbons of the acetates. On the basis of the above observation, the structure **6** was elucidated as 1,3-di-*p*-coumaroyl-6-feruloyl-2′,6′-diacetyl sucrose.

The other compounds isolated were identified as the known smiglaside E,[8] helonioside B,[7] and 2′,6′-diacetyl-3,6-diferuloyl sucrose,[10] by comparing their spectral data with literature values.

Compounds **1**−**6** were evaluated against human oral epithelium carcinoma (KB), human cervical carcinoma (Hela), human colon tumor (DLD-1), human breast adenocarcinoma (MCF-7), human lung carcinoma (A-549), and human medulloblastoma (Med) cells. The bioassay data obtained (Table 3) showed that compounds **4**−**6** exhibited cytotoxicity against DLD-1 cells (ED_{50} = 2.7−5.0 μg/mL), and **1** exhibited weak cytotoxicity against the same cells (ED_{50} = 11.6 μg/mL). As shown in Table 3, most of these phenylpropanoid glycosides, except for **3**, exhibited weak cytotoxicity (ED_{50} = 5.1−13.0 μg/mL) against three to six human tumor cell lines. These results imply that the acetate group in the sucrose unit of these phenylpropanoid glycosides might play a role in mediating cytotoxicity.

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were obtained on a JASCO P-1020 polarimeter. Infrared (IR) spectra were measured on a Mattson Genesis II spectrophotometer (Thermo Nicolet, Madison, WI). NMR spectra were run on Bruker NMR spectrometers (Unity Plus 400 MHz) (Bruker BioSpin GmbH) using CD_3OD as solvent. FABMS data were obtained on a JEOL SX-102A instrument. High-resolution FABMS were measured on a Finnigan/ThermoQuest MAT mass spectrometer. Sephadex LH-20 and silica gel (Merck 70−230 mesh and 230−400 mesh) (Merck) were used for column chromatography, and precoated silica gel (Merck 60 F-254) plates were used for TLC. The spots on TLC were detected by spraying with 5% H_2SO_4 and then heating at 110 °C. HPLC separations were performed on a Shimadzu LC-6AD series apparatus with a SPD-6AV UV detector, equipped with a 250 × 20 mm i.d. preparative Cosmosil AR-II column (Nacalai Tesque, Inc., Kyoto, Japan).

Plant Material. The stems of *Smilax china* (6.0 kg) were collected in the northern mountains of Taiwan, Taipei County, in July 2003 and identified by Professor Mu-Thiung Kao, National Research Institute of Chinese Medicine, Taipei. A voucher specimen (NRICM 2003016) has been deposited in the National Research Institute of Chinese Medicine, Taipei, Taiwan.

Extraction and Isolation. The dried stems of *S. china* were ground and extracted five times with 70% EtOH. The combined EtOH extracts were concentrated to a residue (368 g). After diluting with H_2O, the resulting suspension was partitioned with equal volumes of *n*-hexane and $CHCl_3$. The $CHCl_3$ layer provided 103.0 g of an extract and was separated by silica gel column chromatography eluting with CH_2Cl_2−MeOH (30:1, 25:1, 20:1, 15:1, 10:1, 8:1, 5:1, 3:1, 2:1, 1:1, 1:2, 1:3, 0:1), to yield 13 fractions. After being subjected to cytotoxicity assays against KB, Hela, and DLD-1 cells, fractions 8 and 9 were found to be active [ED_{50} (KB, Hela, and DLD-1) = 15.4, 10.8, and 14.3 μg/mL, respectively, for fraction 8; and 7.9, 8.5, and 7.2 μg/mL, respectively, for fraction 9].

Table 2. ^{13}C NMR Spectroscopic Data for **1**−**6** ($CDCl_3$)[a,b]

C	**1**	**2**	**3**	**4**	**5**	**6**
1	65.7	64.6	66.1	65.7	66.5	66.4
2	105.9	105.6	103.7	104.2	103.3	103.6
3	79.5	78.5	79.3	77.2	79.0	79.5
4	74.7	74.4	74.2	75.8	73.6	74.0
5	82.2	81.0	81.1	79.3	81.1	81.2
6	64.9	65.7	66.1	65.2	65.3	65.3
OAc-4				171.9		
				20.7		
1′	92.7	90.9	93.6	93.9	92.9	90.7
2′	73.0	74.6	73.1	73.0	72.9	74.2
3′	72.5	72.2	75.1	75.0	74.8	72.1
4′	72.5	71.6	71.7	71.3	72.2	71.9
5′	70.0	74.1	74.4	74.5	72.2	72.1
6′	64.4	62.5	62.8	62.4	65.7	65.3
OAc-2′		172.5				172.5
		21.1				21.1
OAc-4′	172.0					
	20.6					
OAc-6′	172.7				173.0	172.9
	20.8				21.0	20.9
1-X′						
α″			147.4	147.4	147.2	147.2
β″			114.8	114.5	115.2	114.6
γ″			168.7	168.4	168.5	168.4
1″			127.2	127.0	127.1	127.1
2″			131.4	131.4	131.3	131.3
3″			117.0	116.8	116.8	116.8
4″			161.3	161.4	161.3	161.4
5″			117.0	116.8	116.8	116.8
6″			131.4	131.4	131.3	131.3
3-X						
α‴	147.9	147.9	148.3	148.6	148.2	148.1
β‴	114.8	114.8	114.8	114.3	114.6	114.2
γ‴	167.8	168.4	168.5	168.0	168.3	168.4
1‴	127.7	127.7	127.7	127.5	127.6	127.1
2‴	111.7	111.8	112.2	112.1	112.0	131.6
3‴	149.4	149.4	149.4	149.4	149.4	116.9
4‴	150.8	150.8	150.8	151.0	150.8	161.6
5‴	124.3	116.5	116.6	116.5	116.4	116.9
6‴	111.7	124.3	124.5	124.6	124.4	131.6
OMe	56.6	56.5	56.6	56.5	56.5	
6-X or X′						
α⁗	147.2	147.2	147.3	147.5	147.2	147.2
β⁗	115.2	115.2	115.2	115.0	115.2	115.2
γ⁗	168.9	169.0	169.2	168.8	168.9	168.9
1⁗	127.7	127.7	127.8	127.6	127.7	127.8
2⁗	112.0	111.9	111.8	111.7	111.6	111.8
3⁗	149.5	149.4	149.4	149.4	149.3	149.4
4⁗	151.0	150.9	150.7	150.7	150.6	150.7
5⁗	116.6	116.5	116.6	116.5	116.5	116.5
6⁗	124.4	124.4	124.4	124.4	124.3	124.3
OMe	56.6	56.5	56.6	56.5	56.5	56.5

[a] Assignments were confirmed by ^1H−^1H COSY, TOCSY, HMBC, and HMQC. [b] Compounds **1**, **2**, and **4**−**6** were measured at 100 MHz, and **3** at 125 MHz.

Table 3. Cytotoxic Activity of Compounds **1**−**6** (ED_{50}, μg/mL)[a]

	cell line					
compound	KB	Hela	DLD-1	MCF-7	A-549	Med
1	11.2	8.9	11.6	10.8	7.1	11.3
2	*b*	*b*	*b*	10.3	12.0	9.4
3	*b*	*b*	*b*	*b*	*b*	*b*
4	13.0	7.3	2.7	*b*	8.4	11.9
5	5.5	5.1	4.5	13.0	12.4	12.2
6	9.0	8.3	5.0	13.0	*b*	*b*
doxorubicin	0.1	0.1	0.1	0.1	0.1	0.1

[a] Key to cell lines used: human oral epithelium carcinoma (KB), human cervical carcinoma (Hela), human colon tumor (DLD-1), human breast adenocarcinoma (MCF-7), human lung carcinoma (A-549), and human medulloblastoma (Med) cells. [b] ED_{50} > 20 μg/mL.

Then, fraction 8 (3.76 g) was chromatographyed on a silica gel column with *n*-hexane−EtOAc [10:1 (1 L), 8:1 (2 L), 6:1 (3 L), 4:1 (2 L), 2:1 (2 L), 1:1 (2 L), EtOAc (2 L)] to give 10 fractions, 8.1−8.10. In turn, fractions 8.4 (203 mg) and 8.5 (156 mg) were further purified by HPLC [C_{18} (particle size, 5 μm) packing column, 250 × 10 mm, MeOH−H_2O, 75:25]. Compounds **1** (2.3 mg), **2** (4.9 mg), and **3** (5.2 mg) were obtained from fraction 8.4, while compounds **7** (3.5 mg), **8** (2.8 mg), and **9** (5.5 mg) were obtained from fraction 8.5. The cytotoxic fraction 9 (3.67 g) was subjected to passage over a silica gel column, eluting with gradient mixture of CH_2Cl_2−MeOH (60:40 to 0:100), to afford 12 fractions, 9.1−9.12. Compounds **4** (5.5 mg), **5** (4.7 mg), and **6** (4.4 mg) were finally purified from fraction 9.6 by HPLC (5 μm C_{18}, 250 × 10 mm, MeOH−H_2O, 70:30).

Smilaside A (1): light yellowish glass; $[\alpha]_D^{24}$ +16.4° (*c* 1.16, MeOH); IR ν_{max} (KBr) 3389, 1741, 1723, 1709, 1630, 1596 cm^{-1}; ^{1}H and ^{13}C NMR data are shown in Tables 1 and 2; ESIMS *m/z* 777 [M − H]$^-$; HRFABMS *m/z* 777.2238 [M − H]$^-$ (calcd 777.2242, $C_{36}H_{41}O_{19}$).

Smilaside B (2): light yellowish powder; $[\alpha]_D^{24}$ +51.9° (*c* 0.27, MeOH); IR ν_{max} (KBr) 3400, 1734, 1721, 1703, 1631, 1595 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 2; ESIMS *m/z* 735 [M − H]$^-$; HRFABMS *m/z* 735.2128 [M − H]$^-$ (calcd 735.2136, $C_{34}H_{39}O_{18}$).

Smilaside C (3): light yellowish powder; $[\alpha]_D^{24}$ +36.1° (*c* 4.71, MeOH); IR ν_{max} (KBr) 3376, 1716, 1698, 1633, 1602 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 2; ESIMS *m/z* 839 [M − H]$^-$; HRFABMS *m/z* 839.2406 [M − H]$^-$ (calcd 839.2399, $C_{41}H_{43}O_{19}$).

Smilaside D (4): light yellowish glass; $[\alpha]_D^{24}$ +39.5° (*c* 2.61, MeOH); IR ν_{max} (KBr) 3390, 1726, 1708, 1630, 1603 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 2; ESIMS *m/z* 881 [M − H]$^-$; HRFABMS *m/z* 881.2515 [M − H]$^-$ (calcd 881.2504, $C_{43}H_{45}O_{20}$).

Smilaside E (5): light yellowish glass; $[\alpha]_D^{24}$ +65.8° (*c* 1.42, MeOH); IR ν_{max} (KBr) 3389, 1731, 1708, 1696, 1630, 1602 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 2; ESIMS *m/z* 881 [M − H]$^-$; HRFABMS *m/z* 881.2496 [M − H]$^-$ (calcd 881.2504, $C_{43}H_{45}O_{20}$).

Smilaside F (6): light yellowish glass; $[\alpha]_D^{24}$ +17.7° (*c* 3.61, MeOH); IR ν_{max} (KBr) 3400, 1742, 1727, 1709, 1631, 1596 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 2; ESIMS *m/z* 893 [M − H]$^-$; HRFABMS *m/z* 893.2491 [M − H]$^-$ (calcd 893.2504, $C_{44}H_{45}O_{20}$).

Smiglaside E (7): light yellowish glass; $[\alpha]_D^{24}$ +52.2° (*c* 3.14, MeOH); IR ν_{max} (KBr) 3390, 1726, 1708, 1630, 1603 cm^{-1}; ESIMS *m/z* 923 [M − H]$^-$.

Helonioside B (8): light yellowish glass; $[\alpha]_D^{24}$ +13.1° (*c* 0.38, MeOH); IR ν_{max} (KBr) 3417, 1721, 1709, 1692, 1630, 1594 cm^{-1}; ESIMS *m/z* 735 [M − H]$^-$.

2′,6′-Diacetyl-3,6-diferuloylsucrose (9): light yellowish glass; $[\alpha]_D^{24}$ +56.6° (*c* 1.75, MeOH); IR ν_{max} (KBr) 3388, 1724, 1708, 1631, 1603 cm^{-1}; ESIMS *m/z* 777 [M − H]$^-$.

Cytotoxicity Assay. MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] agent, against KB, Hela, DLD-1, MCF-7, A-549, and Med cells, was based on the literature[9,10] procedure. In brief, the cells were cultured in RPMI-1640 medium. Test samples were prepared at four concentrations. After these cell lines were seeded in a 96-well microplate for 4 h, 20 μL of sample was placed in each well and incubated at 37 °C for 3 days, and then 20 μL MTT was added for 5 h. After removing the medium and putting DMSO (200 μL/well) into the microplate with shaking for 10 min, the formazan crystals were redissolved and their absorbance was measured on a microtiter plate reader (Dynatech, MR 7000), at a wavelength of 550 nm.

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