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Guaiane Sesquiterpenoids from *Torilis japonica* and Their Cytotoxic Effects on Human Cancer Cell Lines

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A new compound **2** and two known guaiane-type sesquiterpenoids were isolated from the methylene chloride-soluble fraction of the methanolic extract of the fruits of *Torilis japonica* (Umbelliferae) through repeated silica gel and Sephadex LH-20 column chromatography. Their chemical structures were elucidated as torilin (**1**), 11-acetoxy-8-angeloyloxy-1β-hydroxy-4-guaien-3-one (1β-hydroxytorilin, **2**), and 11-acetoxy-8-angeloyloxy-1α-hydroxy-4-guaien-3-one (1α-hydroxytorilin, **3**) by spectroscopic analysis. Compounds **1-3** exhibited cytotoxicity against human A549, SK-OV-3, SK-MEL-2, and HCT15 tumor cells.

Key words: *Torilis japonica,* Umbelliferae, Sesquiterpenoid, 1-Hydroxytorilin

INTRODUCTION

Torilis japonica (Outt.) DC. (Umbelliferae) is widely distributed in Korea, Japan, and China, and has been used as folk medicine to treat impotence, infertility, women's diseases, chronic diarrhea and carbuncle (Lee, 1996; Sung *et al.*, 1998). From the methanolic extract of the fruits, several type of sesquiterpenoids, guaiane, humulene, germacrane and eudesmane, were reported (Itokawa *et al.*, 1983a, 1983b, 1986; Kitajima *et al.*, 1998, 2002; Ryu and Jeong, 2001). In our study on the chemical constituents of this plant, we isolated three guaine-type sesquiterpenoids from a methylene chloride-soluble fraction of the methanolic extract of the fruits of *T. japonica*. The isolated compounds were examined for their cytotoxicity against four human tumor cell lines *in vitro* using a SRB assay. This paper describes the isolation and structural characterization and cytotoxicity of the isolated compounds.

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MATERIALS AND METHODS

General experimental procedure

^{1}H- and ^{13}C-NMR spectra were determined on a JEOL JMN-EX 400 spectrometer in $CDCl_3$. The mass was determined on a Q-TOF (Hybrid tandem mass) mass spectrometer (Applied biosystems, U.S.A.). Optical rotations were obtained using a ADP220 digital polarimeter (Bellingham & Stanley Ltd.). TLC work was carried out using plates coated with silica gel 60 F_{254} (Merck Co.). Silica gel column chromatography was performed on Merck silica gel 60 (230-400 mesh). Sephadex LH-20 was used for the column chromatography (Pharmacia, 25-100 μm). The column used for LPLC was Lobar-A (Merck Lichroprep Si 60, 240-10 mm). All solvents were routinely distilled prior to use. Other chemicals were commercial grade without purification.

Plant materials

The fruits of *T. japonica* were collected and air-dried in June 2003 at Wanju, Chonbuk, Korea. A voucher specimen was deposited in the herbarium of the college of pharmacy, Woosuk University (WSU-03-045).

Extraction and isolation

The shade dried plant material (500 g) was extracted

(three times with MeOH at room temperature) and filtered. The extracts were combined and evaporated *in vacuo* at 40°C. The resultant methanolic extract (55 g) was partitioned with methylene chloride three times to afford a methylene chloride-soluble syrup on drying (20 g). The methylene chloride-soluble extract (7 g) was chromatographed on silica gel column ($CHCl_3$-MeOH, 30:1) to give three fractions (MC1-MC3). Fraction MC1 (3 g) was chromatographed by silica gel column chromatography ($CHCl_3$-MeOH, 35:1) to afford five subfractions (MC11-MC15). Subfraction MC12 (600 mg) was further chromatographed over silica gel (*n*-hexane-EtOAc, 3:1), and purified by Lobar-A column (*n*-hexane-EtOAc, 3:1) to give compound **1** (170 mg). Subfraction MC14 (540 mg) was chromatographed over silica gel (*n*-hexane-EtOAc, 2:1), and purified by Sephadex LH-20 (MeOH) to give compound **2** (80 mg), and subfraction MC15 (280 mg) was chromatographed over silica gel (*n*-hexane-EtOAc, 1:1), and purified by Sephadex LH-20 (MeOH) to give compound **3** (40 mg).

Torilin (1)
Colorless needles (MeOH); m.p. 78 -79; ^{1}H- and ^{13}C-NMR data: See the Table I.

1β-Hydroxytorilin (2)
Colorless oil (MeOH); $[\alpha]_D^{24}$ -2.5° (c 0.1, MeOH); Q-TOF mass $[M+1]^+$ *m/z* 393.2014; ^{1}H- and ^{13}C-NMR data: See the Table I.

1β-Hydroxytorilin (3)
Colorless oil (MeOH); $[\alpha]_D^{24}$ +8.2° (c 0.1, MeOH); ^{1}H- and ^{13}C-NMR data: See the Table I.

Cytotoxicity assay *in vitro*
Sulforhodamin B bioassay (SRB) was used for cytotoxicity evaluation of the isolated compounds against the following four cultured human tumor cell lines: A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian), SK-MEL-2 (skin melanoma), and HCT (colon) (Skehan *et al.*, 1990).

RESULTS AND DISCUSSION

Repeated chromatography of the methylene chloride-soluble fraction of the MeOH extract of the *T. japonica* led to the isolation of three guaiane-type sesquiterpene. Compounds **1-3** have similar patterns in their NMR spectra. ^{1}H- and ^{13}C-NMR data indicated the presence of an acetoxy and an angeloyloxy moiety in the molecules of **1-3**. The main difference of these compounds was ^{13}C-NMR chemical shift values of C-1 (δ 51.6 of **1**, 79.0 of **2**, and 82.7 of **3**). In addition to these evidences, comparison of spectral data with those published in the literature the

Table I. ^{1}H- and ^{13}C-NMR spectral data of compounds **1-3** (in $CDCl_3$)

	1[a]		**2**[b]		**3**[b]	
position	δ_H[mult., *J*(Hz)]	δ_C	δ_H[mult., *J*(Hz)]	δ_C	δ_H[mult., *J*(Hz)]	δ_C
1	2.43 (m)	51.6		79.0		82.7
2	2.60 (dd, 18.4, 6.0)	41.6	2.60 (dd, 18.4, 6.0)	49.4	2.67 (d, 18.0)	51.8
	2.07 (dd, 18.4, 3.0)		2.07 (dd, 18.4, 3.0)		2.48 (d, 18.0)	
3		208.9		206.4		204.2
4		135.4		135.0		137.8
5		174.7		172.8		170.2
6	2.88 (d, 13.6)	26.2	2.68 (d, 13.0)	22.5	2.89 (dd, 18.6, 12.6)	25.6
	2.53 (m)		2.67 (s)		2.65 (d, 18.0)	
7	2.41 (dd, 10.2, 3.5)	46.7	2.41 (m)	46.8	3.45 (dd, 12.6, 4.2)	44.8
8	5.46 (m)	71.1	5.41 (m)	71.0	5.49 (d, 3.0)	71.3
9	2.25 (dd, 14.5, 7.7)	40.9	2.12 (m)	34.5	2.18 (m)	34.9
	1.62 (m)		1.94 (m)		2.05 (m)	
10	1.50 (m)	33.8	1.74 (m)	37.2	2.31 (m)	39.9
11		84.8		84.5		84.3
12	1.52 (s)	25.0*	1.50 (s)	24.5*	1.43 (s)	23.9*
13	1.54 (s)	24.4*	1.52 (s)	24.0*	1.46 (s)	23.5*
14	1.03 (d, 6.5)	23.2	1.07 (d, 6.6)	18.1	0.77 (d, 7.8)	16.3
15	1.73 (s)	8.5	1.70 (s)	7.9	1.72 (s)	7.8
1'		167.1		166.6		166.2
2'		128.1		127.5		126.9
3'	6.09 (m)	138.7	6.07 (m)	138.3	6.07 (m)	139.7
4'	2.01 (d, 7.2)	16.2	1.98 (dd, 7.2, 1.2)	15.8	1.98 (dd, 7.2, 1.8)	15.5
5'	1.91 (s)	21.1	1.89 (s)	20.6	1.79 (s)	20.4
1"		170.7		170.3		169.5
2"	1.99 (s)	23.1	1.96 (s)	22.6	1.96 (s)	22.4

[a]Recorded at 400MHz for ^{1}H and 100 MHz for ^{13}C.
[b]Recorded at 600MHz for ^{1}H and 150 MHz for ^{13}C.

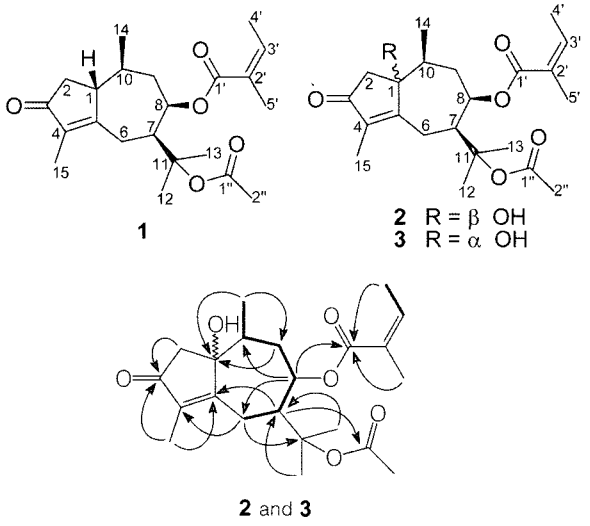

Fig. 1. Structures of compounds **1-3** isolated from *Torlis japonica* and correlations observed in ^{1}H-^{1}H COSY (—) and HMBC (→) spectra of **2** and **3**

structure of **1** established to be torilin, which has been previously isolated from *T. japonica* (Kang *et al.*, 1994; Ryu and Jeong, 2001). Compounds **2** and **3** have very similar patterns in their NMR spectra (^{1}H-, ^{13}C-, ^{1}H-^{1}H COSY, and HMBC). Compounds **2** and **3** were obtained as a colorless oil and compound **2** produced a molecular ion $[M+1]^+$ at *m/z* 393.2014 in it's a Q-TOF mass. The ^{13}C- and DEPT NMR spectra of **2** and **3** showed seven methyl carbons, three methylene carbons, four methine carbons, and five quaternary carbons together with one ketonic and two ester carbonyls. The ^{1}H-^{1}H and ^{1}H-^{13}C COSY spectral data along with the above evidences led to the partial structures of C_6-C_7-C_8-C_9-C_{10}-C_{14}, the acetoxy and the angeloyloxy groups. In the HMBC spetrum of **2**, the carbon signal C-1 (δ 79.0) showed a ^{1}H-^{13}C long-range correlation with H-9 (δ 2.12 and 1.94), and H-14 (δ 1.07). The carbon signal C-1' (δ 166.6) of the angelate substituent correlated with H-8 (δ 5.41), and C-1" (δ 170.3) of the acetate correlated with H-7 (δ 2.41). The relative stereochemistry of C_7-C_8 and C_{10} was determined to be similar to that of **1**, torilin, on the basis of the coupling constants observed in the ^{1}H-NMR spectrum. Furthermore, the stereochemistry of C_7-C_8 and C_{10} was confirmed from its 2D NOESY spectrum. From the above data, the structure of **2** was deduced 1-hydroxytorilin. The correlation patterns of the HMBC and NOESY spectra of **3** were very similar that of **2**. Therefore, compounds **2** and **3** have the same structure except for the difference of the stereochemistry of hydroxyl group of C-1. A literature survey revealed that 1α-hydroxytorilin ($[\alpha]_D^{24}$ +12.4) was reported from *Cnidium monnieri* (Oh *et al.*, 2002). The optical rotation of **3** was obtained as a positive value (+8.2°), and the NMR spectral data were in well accord

Table II. Cytotoxicity of compounds **1-3** against human tumor cell lines (ED_{50}, μg/mL)[a]

Compounds	Cell lines[b]			
	A549	SK-OV-3	SK-MEL-2	HCT15
1	6.54	13.75	5.16	14.75
2	20.29	14.74	19.89	40.33
3	20.52	18.24	17.48	42.54
Doxorubicin	0.008	0.072	0.009	0.101

[a] ED_{50} was defined as a concentration (μg/mL) that caused 50% inhibition of cell growth *in vitro*.

[b] Cell lines: A-549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; HCT15, human colon cancer

with 1α-hydroxytorilin reported in the literature. On the basis of optical rotation value and comparison of NMR data, structure of **3** was identified as 11-acetoxy-8-angeloyloxy-1α-hydroxy-4-guaien-3-one (1α-hydroxytorilin). On the other hand, optical rotation of **2** was obtained as a negative value (-2.5°). From the ^{13}C-NMR spectrum of **2**, the chemical shift value of C-1 (d 79.0) was different from that of compound **3** (δ 82.7). Consequently, with above evidences, the structure of **2** was deduced as 11-acetoxy-8-angeloyloxy-1β-hydroxy-4-guaien-3-one (1β-hydroxytorilin). Torilin (**1**) have been reported to have testosterone 5α-reductase inhibitory (Park *et al.*, 2003), anticancer (Kim *et al.*, 2000) and anti-inflammatory activities (Lee *et al.*, 1999), and the ability to reverses multidrug-resistance in cancer cells (Kim *et al.*, 1998). To our best knowledge, this is the first report on the isolation of compound **2** from the nature.

The cytotoxicity of the compounds was tested by SRB (Sulforhodamin B) assay method against four cultured human tumor cells. Compounds **1-3** showed moderate cytotoxicity against the human cancer cells (Table II).

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