

## A New Guaiane Type Sesquiterpene from *Torilis japonica*

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A new guaiane type sesquiterpene was isolated from the fruit of *Torilis japonica* (Umbelliferae). Based on NMR, IR and mass spectroscopy its structure was confirmed as deangeloyloxy torilin,  $1\beta$ ,  $7\alpha$ ,  $10\alpha$ H-11-acetoxy-guaia-4-en-3-one (**1**). This is the first report showing that this compound can be isolated from *Torilis japonica*.

**Key words:** *Torilis japonica*, Umbelliferae, Seeds, Guaiane, Sesquiterpene

### INTRODUCTION

*Torilis japonica* (Houtt.) DC is a biennial plant that is found widely in East Asia. The fruits of this plant (Umbelliferae, Korean name "Sasangja") have been used as a crude drug in traditional Chinese medicine for the treatment of itching, eczema and impotence. Itokawa *et al.* (1983) reported some antispasmodic activity of the ethanol extract of *Torilis japonica*. Torilin from this plant has been reported to have anti-inflammatory (Lee *et al.*, 1999) and anticancer activity including anti-angiogenic activity (Kim *et al.*, 2000), the ability to reverse multidrug-resistance in cancer cells (Kim *et al.*, 1999) and possess anti-invasive activity in human fibrosarcoma cells (Kim *et al.*, 2001). The chemical constituents of *Torilis japonica* contain many sesquiterpenoids including torilin (Chikamatsu *et al.*, 1969; Kang *et al.*, 1994; Itokawa *et al.*, 1986), essential oils (Fujita, 1990) and hemiterpenoids (Kitajima *et al.*, 1998).

In the course of our ongoing research in identifying anti-inflammatory agents from plant sources, a new guaiane type sesquiterpene was isolated from *Torilis japonica* and its structure was confirmed by spectroscopic methods.

### MATERIALS AND METHODS

#### General

The nuclear magnetic resonance (NMR) spectra were

obtained on a Bruker AMX-400 NMR spectrometer and the  $^1\text{H}$  chemical shifts were referenced to residual undeuterated solvent peak of  $\text{CDCl}_3$ . The electron impact (EI) mass spectra were taken with a direct inlet and recorded on a JMS-DX 303 mass spectrometer (JEOL). The infrared (IR) spectra were recorded on a Jasco FT-IR-430 spectrometer in a  $\text{CHCl}_3$  solution. The ultra-violet (UV) spectra were obtained on a Pharmacia Biotech Ultraspec 4000 UV-VIS spectrophotometer and the optical rotation was determined on a Jasco DIP-1000 digital polarimeter.

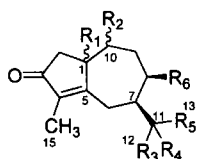
#### Plant material

The *T. japonica* fruit was purchased from a Kyungdong oriental drug market in Seoul and authenticated by Prof. T. H. Kim at the College of Pharmacy, Sookmyung Women's University. A voucher specimen (No. SPH 99008) was deposited in the herbarium of the Sookmyung Women's University.

#### Extraction and isolation

The MeOH extracts from the dried plant materials (2 kg) were obtained after soaking the fruits at room temperature for 7 days. The solvent was removed by evaporation yielding a syrupy product (159 g), which was suspended in  $\text{H}_2\text{O}$  and partitioned successively with hexane and EtOAc to give hexane (22 g) and EtOAc (128 g) soluble fractions. Five column-fractions (EJ-1~EJ-5) were obtained by silicagel column chromatography of the hexane-soluble fraction using  $\text{CHCl}_3$  as the eluent. Torilin was purified from fraction EJ-2 and EJ-1 (11.5 g) containing compound **1**, and was further chromatographed on silica-gel by eluting with *n*-hexane- $\text{CHCl}_3$  (1:1) and hexane-acetone (30:1), and finally purified by reversed-phase

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Substituents	1	2	3	4	5
R <sub>1</sub>	β-H	β-H	α-H	α-H	α-H
R <sub>2</sub>	β-CH <sub>3</sub>	β-CH <sub>3</sub>	β-CH <sub>3</sub>	β-CH <sub>3</sub>	β-CH <sub>3</sub>
R <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>2</sub>
R <sub>4</sub>	O-Ac <sup>a</sup>	O-Ac	OH	H	-
R <sub>5</sub>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	COOH	CH <sub>3</sub>
R <sub>6</sub>	H	β-O-Ang <sup>b</sup>	H	H	H

<sup>a</sup>Ac=acetyl; <sup>b</sup>Ang=angeloyl

HPLC ( $\mu$ -Bondapak C-18 column, 300 × 10 mm; 40% CH<sub>3</sub>CN 2 ml/min; UV 254 nm) to yield compound **1** (5.8 mg). The purity of compound **1** was confirmed by HPLC ( $\mu$ -Bondapak C-18 column, 300 × 3.9 mm; 40% CH<sub>3</sub>CN 1 ml/min; UV 254 nm) as a single peak.

### Compound 1

colorless oil;  $[\alpha]_D^{29} = -31.8^\circ$  (CHCl<sub>3</sub>, c 0.17); IR  $\nu_{\max}^{\text{CHCl}_3}$  cm<sup>-1</sup>: 1730, 1699 and 1642; UV  $\lambda_{\max}^{\text{EtOH}}$  nm (log  $\epsilon$ ): 240.0 (4.165); EIMS (probe) 60eV,  $m/z$  (rel. int.): 278 [M]<sup>+</sup> (0.2) (calc. for C<sub>17</sub>H<sub>26</sub>O<sub>3</sub>), 218 [M-HOAc] (100), 203 [218-CH<sub>3</sub>] (31), 175 [218-C<sub>2</sub>H<sub>5</sub>CO] (63), 161 [218-C<sub>3</sub>H<sub>7</sub>CO] (37), 147 (36), 121 (16), 91 (30), 59 (44); <sup>1</sup>H NMR (see Table I); <sup>13</sup>C NMR (see Table II)

## RESULTS AND DISCUSSION

The hexane-soluble fraction of the methanol extract of

*T. japonica* was chromatographed on silica gel and reversed-phase HPLC to give compound **1** together with torilin (Chikamatsu *et al.*, 1969) and 1 $\beta$ , 6 $\alpha$ -dihydroxyeudesm-4(15)-ene (Bohlmann *et al.*, 1983). The IR spectrum of compound **1** showed strong bands at 1730 cm<sup>-1</sup> indicating the presence of acyl carbonyl carbon, and 1699 and 1642 cm<sup>-1</sup> indicating the presence of a cyclopentenone ring in the structure of compound **1**. The <sup>1</sup>H-NMR spectra of compound **1** showed three *tert*-methyl protons (1.47, 1.48 and 1.70 ppm) and one *sec*-methyl protons (1.04 ppm). The <sup>13</sup>C-NMR spectra showed four methylene carbons (26.8, 28.9, 32.8 and 42.3 ppm), three methine carbons (39.6, 44.1 and 51.2 ppm), one tetrasubstituted double bond (136.0 and 170.7 ppm), one carbonyl carbon (209.3 ppm), one acetoxy group (22.4 and 176.3 ppm), and one oxygenated quaternary carbon (85.7 ppm) as shown in Table II. From the distinctive chemical shift of an olefinic carbon at 170.7 ppm, the position of the double bond was determined to be an exocyclic bond of the heptacyclic ring, which is common in guaiane-type sesquiterpenoids.

The overall pattern of the NMR spectra is quite similar to the spectrum of torilin (Kang *et al.*, 1994), except for the absence of an oxyangeloyl group at the C-8 position. The oxymethine carbon peak at 70.2 ppm for torilin was replaced by a methylene carbon at 28.9 ppm in the DEPT spectra of compound **1**. The presence of an acetyl group at C-11 can be deduced from the downfield shift of the <sup>1</sup>H NMR peaks of the 12- and 13-methyl groups. These were found at approximately 1.2 ppm with a hydroxy group at C-11 position of compound **3** (Jakupovic *et al.*, 1987). Therefore, acetyl group in compound **1** was confirmed to reside on C-11, which is an oxygenated quaternary carbon. Therefore, the structure of compound **1**

**Table I.** <sup>1</sup>H NMR data of compound **1** and related compounds

Proton	1 <sup>a</sup>	2 <sup>b</sup>	3 <sup>c</sup>	5 <sup>d</sup>
1	2.29 (m)	2.43 (m)	3.04 (br. d 7)	3.13 (dddq)
2	2.03 (dd 18.5, 2.5)	2.06 (dd 18.5, 3.4)	1.96 (d 19)	2.04 (ddd)
	2.60 (dd 18.5, 6.3)	2.60 (dd 18.5, 6.4)	2.47 (dd 19, 7)	2.58 (ddd)
6	2.14 (m)	2.53 (br dd 13.6, 10.4)	2.10 (br dd 20, 13)	2.77 (br d)
	2.84 (br d 9.9)	2.87 (d 13.8)	2.99 (br d 20)	2.95 (br dd)
7	2.14 (m)	2.42 (dd 10.4, 3.7)	1.62 (m)	2.32 (br dd)
8	1.62 (m)	5.46 (br ddd 8.4, 7.7, 3.7)		1.58 (m)
9	1.62 (m)	-		1.75 (m), 1.84 (m)
10	1.27 (m)	1.50 (m)	2.00 (m)	2.11 (dddq)
12	1.47 (s)	1.52 (s)	1.15 (s)	4.75 (br s), 4.70 (br s)
13	1.48 (s)	1.54 (s)	1.19 (s)	1.77 (br s)
14	1.04 (d 6.7)	1.03 (d 6.7)	0.53 (d 7)	0.65 (d)
15	1.70 (d 1.9)	1.73 (d 2.0)	1.60 (br s)	1.66 (br s)
Ac	2.00 (s)	1.99 (s)	-	-

<sup>a</sup>In CDCl<sub>3</sub>, 400MHz. Coupling constants (*J*) are in Hz.

<sup>b</sup>In CDCl<sub>3</sub>, 300MHz. Data from Kang *et al.* (1994).

<sup>c</sup>In CDCl<sub>3</sub>, 400MHz. Data from Jakupovic *et al.* (1987).

<sup>d</sup>In CDCl<sub>3</sub>, 400MHz. Data from Zdero *et al.* (1989).

**Table II.**  $^{13}\text{C}$  NMR data of compound **1** and related compounds

Carbon	<b>1</b> <sup>a</sup>	<b>2</b> <sup>b</sup>	<b>4</b> <sup>c</sup>	<b>5</b> <sup>d</sup>
1	51.2 (d)	51.0	45.5	44.6
2	42.3 (t)	41.2	40.7	38.0
3	209.3 (s)	207.9	208.8	208.2
4	136.0 (s)	135.2	137.2	137.7
5	170.7 (s)	173.6	175.9	175.2
6	26.8 (t)	25.7	29.4	35.4
7	44.1 (d)	46.4	38.4	46.0
8	32.8 (t)	70.7	27.6	31.4
9	28.9 (t)	40.6	38.4	36.8
10	39.6 (d)	33.5	36.0	41.4
11	85.7 (s)	84.4	45.0	150.3
12	23.9 (q)	23.9	11.5	109.2
13	23.5 (q)	24.5	179.0	20.2
14	22.9 (q)	22.6	12.1	12.1
15	8.1 (q)	8.0	7.3	8.0
Ac	22.4 (q)	22.5	-	-
	176.3 (s)	170.2	-	-

<sup>a</sup>In  $\text{CDCl}_3$ , 100MHz. Carbon multiplicity were determined by DEPT experiments. s=quaternary, d=methines, t=methylene, q=methyl carbons.

<sup>b</sup>In  $\text{CDCl}_3$ , 75MHz. Data from Kang *et al.* (1994).

<sup>c</sup>In  $\text{CDCl}_3$ , 100MHz. Data from Miski *et al.* (1987).

<sup>d</sup>In  $\text{CDCl}_3$ , 100MHz. Data from Zdero *et al.* (1989).

was confirmed to be a deangeloyloxy torilin and an acetate of compound **3**. In order to accomplish the relative stereochemistry around C-1 and C-10,  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of other closely related compounds (compounds **1-5**) with a guaiane-type skeleton were compared. As shown in Table I and Table II, the chemical shifts of H-1, H-10, H-14 and C-1, C-14 showed characteristic values according to the C-1 and C-10 configurations (Kang *et al.*, 1994; Jakupovic *et al.*, 1987; Miski *et al.*, 1987; Zdero *et al.*, 1989). For compounds **4** and **5**, the chemical shifts of C-1 and C-14 were approximately 45 ppm and 12 ppm, respectively. These values were shifted down field compared to those of compounds **1** and **2**. This may be the result of the different configurations at C-1 and C-10, which is observed in many guaiane-type sesquiterpenoids. The NMR data from compound **1** corresponded well to that of torilin. Based on this similarity the relative configurations at H-1 and H-10 were suggested to be  $\beta$  and  $\alpha$ , respectively. Their configurations were confirmed by nuclear Overhauser effect (NOE) experiments (Zdero *et al.*, 1989; Zdero *et al.*, 1988). A NOE interaction was found between H-1 and H-14 in the nuclear Overhauser and exchange spectroscopy (NOESY) spectrum of compound **1**. The H-7 configuration was estimated to be  $\alpha$  as is found all sesquiterpenes from the Umbelliferae family with a known stereochemistry. The structure of compound **1** was confirmed to be deangeloyloxy torilin,  $1\beta$ ,  $7\alpha$ ,  $10\alpha$ H-11-acetoxy-guaia-4-en-3-one, which is the first report of this compound being isolated from this plant.

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