

Cytotoxic β -Dihydroagarofuran Sesquiterpenoids from the Fruits of *Celastrus orbiculatus*

Jing Xu^a, Yuan-Qiang Guo^{a,*}, Xian Li^{b,*}, Kun Wei^c, Xiaojun Zhao^d, and Shuzhong Zhang^e

^a Department of Pharmaceutical Sciences, Nankai University, Tianjin 300071, P.R. China. E-mail: victgyq@163.com

^b Research Department of Natural Medicine, Shenyang Pharmaceutical University, Shenyang 110016, P.R. China

^c School of Chemical Science and Technology, Yunnan University, Yunnan 650091, P.R. China

^d College of Chemistry & Life Sciences, Tianjin Normal University, Tianjin 300387, P.R. China

^e State Key Laboratory of Elemento-organic Chemistry, Nankai University, Tianjin 300071, P.R. China

* Authors for correspondence and reprint requests

Z. Naturforsch. **63c**, 515–518 (2008); received February 4, 2008

Assay-guided fractionation led to the isolation of nine β -dihydroagarofuran sesquiterpenoids from the fruits of *Celastrus orbiculatus*. All isolated β -dihydroagarofuran sesquiterpenoids were tested for their cytotoxic activity against human melanoma A375-S2 and human cervical carcinoma Hela cell lines. Among them, compounds **1–5** and **7** showed cytotoxic activity. Compound **3** exhibited promising cytotoxicity against both human melanoma A375-S2 and human cervical carcinoma Hela cell lines. The structure-activity relationship was discussed briefly.

Key words: *Celastrus orbiculatus*, Sesquiterpenoids, Cytotoxic Activity

Introduction

Celastrus orbiculatus Thunb. (Celastraceae), which is widely distributed in China, has been used as a folk medicine in China for tranquilization and hypnogenesis (Editorial Commission of Traditional Chinese Medicine, 1999). Previous literature reported the main constituents of sesquiterpenoids isolated from this plant and their antifeedant, anti-inflammatory activity, and multi-drug resistance (Editorial Commission of Traditional Chinese Medicine, 1999; Wang and Chen, 1995; Kim *et al.*, 1998, 1999; Jin *et al.*, 2002; Guo *et al.*, 2003, 2004, 2005a, b). In addition, several flavonones from this plant and their anti-inflammatory activity have also been reported (Min *et al.*, 1999; Hwang *et al.*, 2001). However, up to the present, there has been no report on β -dihydroagarofuran sesquiterpenoids of *C. orbiculatus* against human melanoma A375-S2 and human cervical carcinoma Hela cell lines.

In our intended research, assay-guided fractionation led to the isolation of nine β -dihydroagarofuran sesquiterpenoids: $1\beta,6\alpha$ -diacetoxy- 9α -ben-

zoyloxy- β -dihydroagarofuran (**1**), $1\beta,6\alpha,8\beta$ -triacetoxy- 9α -benzoyloxy- β -dihydroagarofuran (**2**), 1β -acetoxy- $6\alpha,9\alpha$ -dibenzoyloxy- β -dihydroagarofuran (**3**), $1\beta,6\alpha,13$ -triacetoxy- 9α -benzoyloxy- β -dihydroagarofuran (**4**), $1\beta,6\alpha$ -diacetoxy- $8\beta,9\beta$ -dibenzoyloxy- β -dihydroagarofuran (**5**), $1\beta,6\alpha$ -diacetoxy- 9β -benzoyloxy- 8β -cinnamoyloxy- β -dihydroagarofuran (**6**), 6α -acetoxy- $1\beta,8\beta,9\beta$ -tribenzoyloxy- β -dihydroagarofuran (**7**), $1\beta,2\beta,6\alpha$ -triacetoxy- 9α -cinnamoyloxy- β -dihydroagarofuran (**8**), $1\beta,2\beta,13$ -triacetoxy- 9α -cinnamoyloxy- β -dihydroagarofuran (**9**). All isolated β -dihydroagarofuran sesquiterpenoids were tested for cytotoxic activity against human melanoma A375-S2 and human cervical carcinoma Hela cell lines *in vitro* using the MTT assay.

Materials and Methods

Plant material

The fruits of *Celastrus orbiculatus* were collected in Shenyang, Liaoning Province, P. R. China and identified by Prof. Yunzhen Guo, Shenyang Pharmaceutical University, Shenyang, P. R. China.

A voucher specimen (No. 200315) was deposited at the Research Department of Natural Medicine, Shenyang Pharmaceutical University.

Extraction and isolation

The air-dried fruits of *Celastrus orbiculatus* (10 kg) were extracted with 95% ethanol under reflux for 2 h. After removing the solvent *in vacuo*, the ethanol extract was suspended in water, and then partitioned with petroleum ether, chloroform, EtOAc and *n*-BuOH successively. The petroleum ether-soluble part exhibited the most cytotoxic activity among these partitions. The petroleum ether part (160 g) was subjected to column chromatography on silica gel (200–300 mesh) and eluted with petroleum ether/acetone (100:0 – 1:1, v/v) to provide 9 fractions (Frs. 1–9). Fr. 3 was followed by column chromatography using a semi-preparative Shimadzu CTO-6A HPLC instrument equipped with a Shimadzu Shim-pack PREP-ODS (250 × 21.6 mm i.d.) column (flow rate 5 ml/min, UV 254 nm) to yield compounds **1–5** using MeOH/H₂O (80:20) as eluent. The purification of Fr. 5 using the same HPLC procedure as above with the solvent MeOH/H₂O (72:28) resulted in the isolation of compounds **6–9**.

Methods of chromatography and structure analysis

Silica gel for chromatography was purchased from Qingdao Ocean Chemical Group Co., Qingdao, P. R. China. Semi-preparative HPLC separations were performed on the above HPLC system. ¹H and ¹³C NMR spectra were obtained on a Bruker-ARX-300 spectrometer in CDCl₃, using TMS as internal standard. ESI-MS was performed on a VG-70SE mass spectrometer (VG, Danvers, USA).

Determination of cytotoxic activity

The cells were grown and maintained in a humidified incubator at 37 °C and in 5% CO₂ atmosphere in RPMI 1640 medium containing 100 g/L fetal bovine serum (FBS) and 10 g/L penicillin-streptomycin-glutamine (PSG). The MTT assay, which is based on the reduction of MTT [3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide] by mitochondrial dehydrogenase to a purple formazan product (Mosmann, 1983), was used to assess the antiproliferative action of the fruit extracts, fractions and pure compounds of *Celastrus*

orbiculatus in human melanoma A375-S2 and human cervical carcinoma Hela cells. The cells were suspended at a density of 2 · 10⁴ cells/mL in RPMI 1640 medium containing 100 g/L FBS and 10 g/L PSG. Then 100 μL each were seeded into each well of 96-well microtiter plates and incubated for 24 h. Different extracts and pure compounds with various concentrations were added to the wells and incubated at 37 °C in 5% CO₂ for 48 h. MTT solution (10 μL) of 0.5 mg/mL was then added to each well and incubated for another 4 h. After removing the upper solution, the resulting MTT-formazan product was dissolved by the addition of 100 μL DMSO solution into each well, followed by mixing and measuring the absorbance at 570 nm using a microplate reader (Bio-Rad, Model 550, Hercules, USA). The results are expressed as the optical density ratio of the treated to control samples: cells inhibition = [(control absorbance – sample absorbance) / control absorbance] · 100%.

Results and Discussion

In our screening, the ethanol extract of the fruits of *Celastrus orbiculatus* was found to exhibit significant cytotoxic activity against Hela and A375-S2 cell lines. To isolate the cytotoxic constituents from the ethanol extract of fruits of *C. orbiculatus*, the petroleum ether-soluble part was subjected to chromatography, as described in Materials and Methods. Nine compounds, **1–9**, were isolated by activity-guided fractionation. Their ¹H, ¹³C NMR and ESI-mass spectra were recorded for structure elucidation. By comparing their spectral data with those reported in the literature, compounds **1–9** were established to be 1β,6α-diacetoxy-9α-benzoyloxy-β-dihydroagarofuran (**1**) (Guo *et al.*, 2003), 1β,6α,8β-triacetoxy-9α-benzoyloxy-β-dihydroagarofuran (**2**) (Guo *et al.*, 2003), 1β-acetoxy-6α,9α-dibenzoyloxy-β-dihydroagarofuran (**3**) (Takahashi *et al.*, 1993), 1β,6α,13-triacetoxy-9α-benzoyloxy-β-dihydroagarofuran (**4**) (Guo *et al.*, 2005b), 1β,6α-diacetoxy-8β,9β-dibenzoyloxy-β-dihydroagarofuran (**5**) (Guo *et al.*, 2004), 1β,6α-diacetoxy-9β-benzoyloxy-8β-cinnamoyloxy-β-dihydroagarofuran (**6**) (Tu *et al.*, 1991), 6α-acetoxy-1β,8β,9β-tribenzoyloxy-β-dihydroagarofuran (**7**) (Guo *et al.*, 2004), 1β,2β,6α-triacetoxy-9α-cinnamoyloxy-β-dihydroagarofuran (**8**) (Guo *et al.*, 2005b), 1β,2β,13-triacetoxy-9α-cinnamoyloxy-

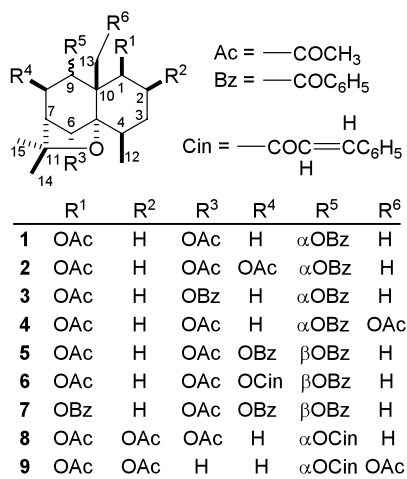


Fig. 1. Chemical structures of compounds **1–9** isolated from the fruits of *Celastrus orbiculatus*.

β-dihydroagarofuran (**9**) (Guo *et al.*, 2005a) (Fig. 1), respectively.

The cytotoxic activities of the isolated compounds were determined using the MTT assay method against two cultured human melanoma A375-S2 and human cervical carcinoma Hela cell lines. As shown in Table I, compounds **1**, **2**, **3**, and **7** exhibited promising cytotoxicity against the Hela cells with IC₅₀ values of 19.0, 82.2, 2.9, 68.9 μM, respectively. Concerning the A375-S2 cell line, compounds **1**, **3**, **4** and **5** inhibited the cell proliferation with IC₅₀ values of 48.8, 7.3, 37.9, 77.6 μM, respectively. The cytotoxic activity of all active sesquiterpenoids **1–5** and **7** seemed to be dose-dependent; the proliferation of Hela cells or A375-S2 cells was more inhibited when the concentration of every compound increased. Compounds **4**, **5**, **6**, **8** and **9** showed very weak activities against Hela cells, while compounds **2**, **6**, **7**, **8** and **9** were considered inactive against A375-S2 cells, due to their IC₅₀ values > 100 μM. According to the above IC₅₀ values, compound **3** showed the highest cytotoxicity against either Hela cells or A375-S2 cells.

The above pharmacological data show the different cytotoxic activities of sesquiterpenoids from

Table I. IC₅₀ values of compounds **1–9** isolated from the fruits of *Celastrus orbiculatus* Thunb. against human cervical carcinoma Hela and human melanoma A375-S2 cell lines.

Compound	IC ₅₀ [μM]	
	Hela	A375-S2
1	19.0	48.8
2	82.2	–
3	2.9	7.3
4	–	37.9
5	–	77.6
6	–	–
7	68.9	–
8	–	–
9	–	–
5-Fluorouracil	22.6	5.0

IC₅₀ values refer to the 50% inhibition concentration (μM) and were calculated from regression lines using five different concentrations with triplicate determinations. 5-Fluorouracil, positive control.
– IC₅₀ > 100 μM.

C. orbiculatus. By analysis of the structures and activities of compounds **1–9** against Hela cells, the acetoxy group at C-1, the benzoyloxy group at C-9 and different substituent groups at C-6 led to different cytotoxic activity, and the presence of the benzoyloxy group at C-6 showed the most activity, while the increasing substituent groups at C-2 or C-8 led to more weak activity. Concerning the structure-activity relationship of these sesquiterpenoids against A375-S2 cells, it is nearly the same to the above results, but the acetoxy group at C-13 led to more cytotoxic activity, which was verified by comparison the structures of compounds **1** and **4**. The cytotoxic activity of the ethanol extract of fruits of *C. orbiculatus* against human melanoma A375-S2 and human cervical carcinoma Hela cells due to its content of sesquiterpenoids suggested its potential therapeutic value for the cancer treatment.

Acknowledgements

This work was supported in part by the Scientific & Technological Innovation Fund of Nankai University, P. R. China.

- Editorial Commission of Traditional Chinese Medicine, State Administration of Traditional Chinese Medicine (1999), *Traditional Chinese Medicine*, Vol. 5. Shanghai Science & Technology Press, Shanghai, p. 166.
- Guo Y. Q., Li X., Wang J. H., Li W., and Sha Y. (2003), A new sesquiterpene ester from the fruits of *Celastrus orbiculatus*. *J. Asian Nat. Prod. Res.* **5**, 205–208.
- Guo Y. Q., Li X., Xu J., Li N., Meng D. L., and Wang J. H. (2004), Sesquiterpene esters from the fruits of *Celastrus orbiculatus*. *Chem. Pharm. Bull.* **52**, 1134–1136.
- Guo Y. Q., Li X., Ma Z. J., Cui W. S., Wang J. H., and Ju Z. X. (2005a), A new sesquiterpene ester from the fruits of *Celastrus orbiculatus*. *J. Asian Nat. Prod. Res.* **7**, 157–160.
- Guo Y. Q., Li X., Xu J., Meng D. L., Li Y. S., and Ohizumi Y. (2005b), Sesquiterpene esters from the fruits of *Celastrus orbiculatus*. *Chem. Lett.* **34**, 764–765.
- Hwang B. Y., Kim H. S., Lee J. H., Hong Y. S., Ro J. S., Lee K. S., and Lee J. J. (2001), Antioxidant benzoylated flavan-3-ol glycoside from *Celastrus orbiculatus*. *J. Nat. Prod.* **64**, 82–84.
- Jin H. Z., Hwang B. Y., Kim H. S., Lee J. H., Kim Y. H., and Lee J. J. (2002), Antiinflammatory constituents of *Celastrus orbiculatus* inhibit the NF-kappaB activation and NO production. *J. Nat. Prod.* **65**, 89–91.
- Kim S. E., Kim Y. H., Lee J. J., and Kim Y. C. (1998), A new sesquiterpene ester from *Celastrus orbiculatus* reversing multidrug resistance in cancer cells. *J. Nat. Prod.* **61**, 108–111.
- Kim S. E., Kim H. S., Hong Y. S., Kim Y. C., and Lee J. J. (1999), Sesquiterpene esters from *Celastrus orbiculatus* and their structure-activity relationship on the modulation of multidrug resistance. *J. Nat. Prod.* **62**, 697–700.
- Min K. R., Hwang B. Y., Lim H. S., Kang B. S., Oh G. J., Lee J., Kang S. H., Lee K. S., Ro J. S., and Kim Y. (1999), (-)-Epiatzelechin: cyclooxygenase-1 inhibitor and anti-inflammatory agent from aerial parts of *Celastrus orbiculatus*. *Planta Med.* **65**, 460–462.
- Mosmann T. (1983), Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **65**, 55–63.
- Takaishi Y., Ohshima S., Nakano K., Tomimatsu T., Tokuda H., Nishino H., and Iwashima A. (1993), Structures of sesquiterpene polyol esters from *Celastrus stephanotiifolius* with potential tumor-promotion inhibitor activity. *J. Nat. Prod.* **56**, 815–824.
- Tu Y. Q., Wu T. X., Li Z. Z., Zhen T., and Chen Y. Z. (1991), Sesquiterpene polyol esters from *Celastrus paniculatus*. *J. Nat. Prod.* **54**, 1383–1386.
- Wang M. A. and Chen F. H. (1995), Two new sesquiterpenes from *Celastrus orbiculatus*. *Chin. Chem. Lett.* **6**, 229–230.